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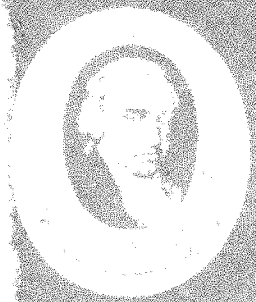

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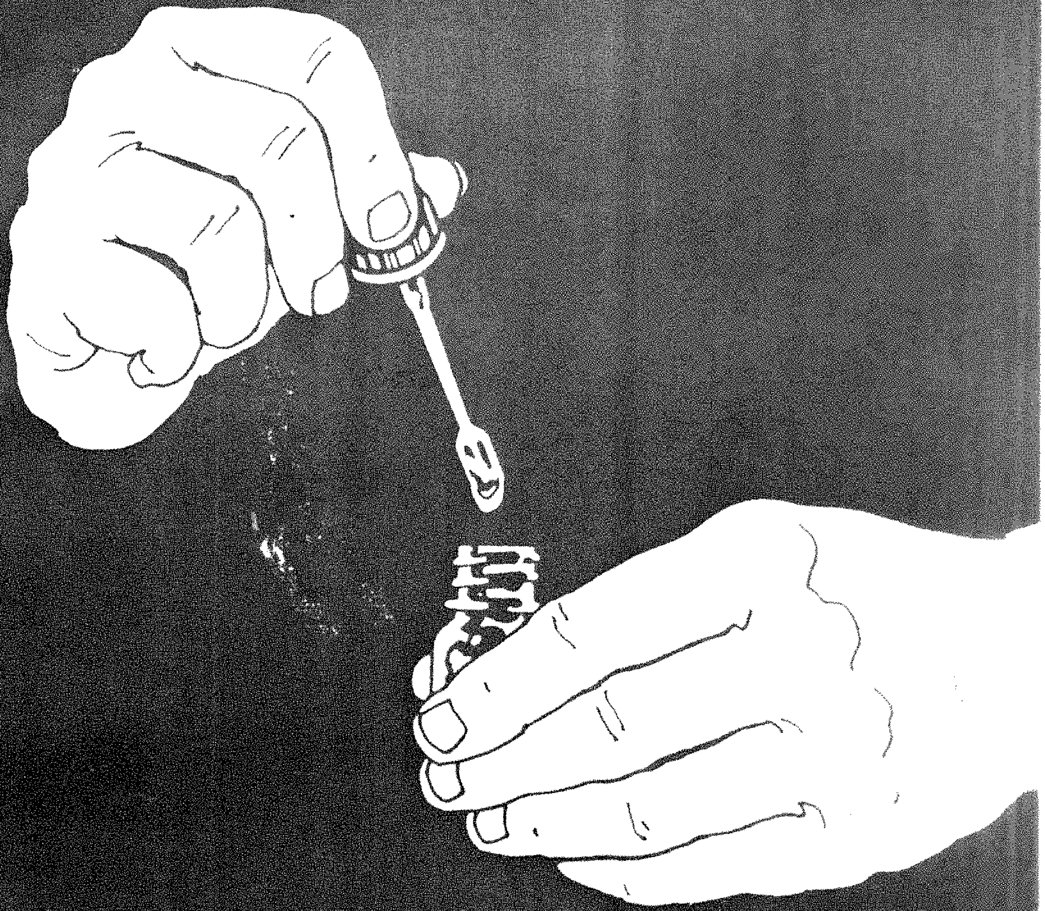

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Refs: Practitioner, 207, 197, 1971.
Practitioner, 209, 453, 1972.

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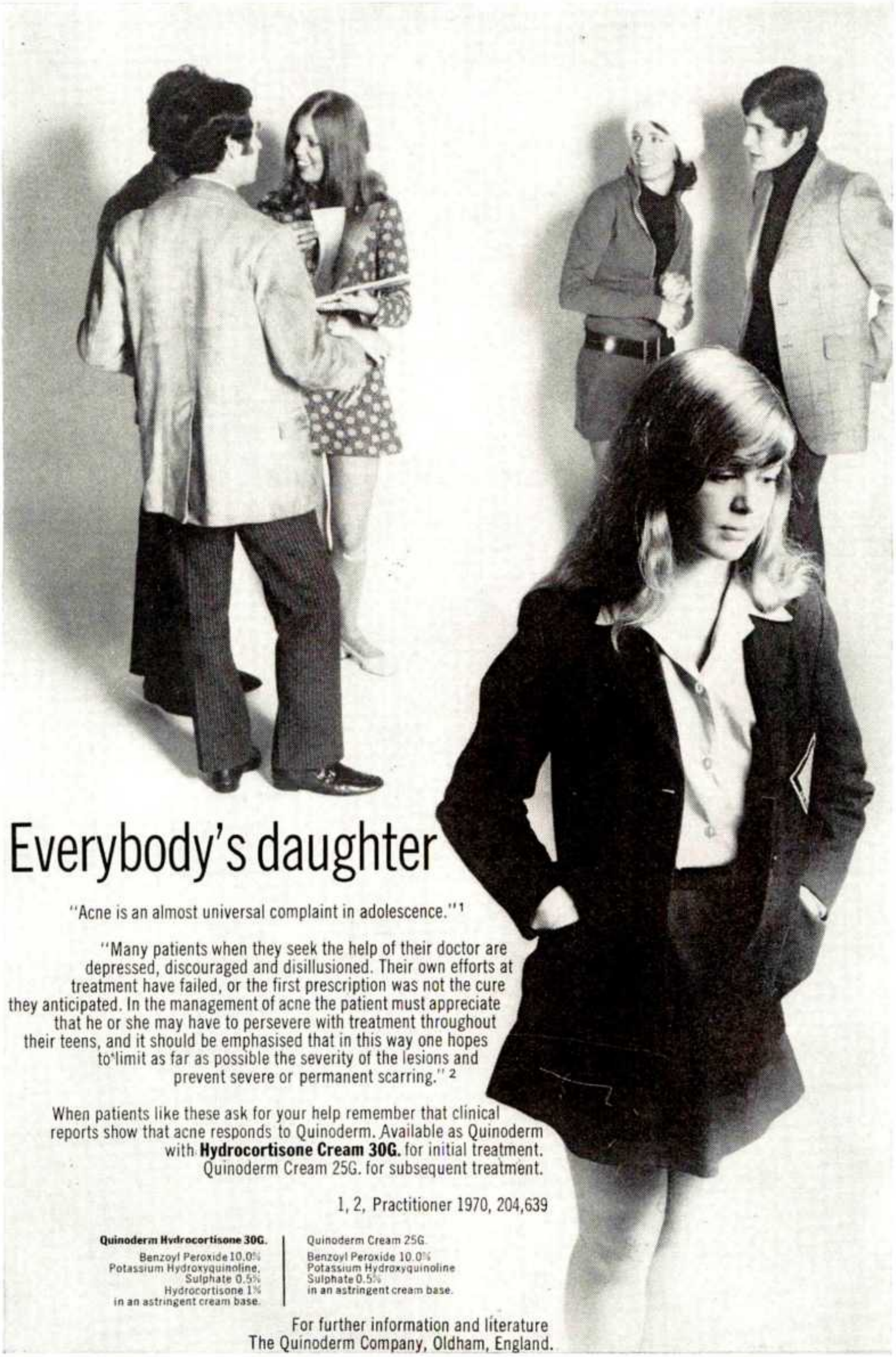

Everybody's daughter

"Acne is an almost universal complaint in adolescence."[1]

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1, 2, Practitioner 1970, 204,639

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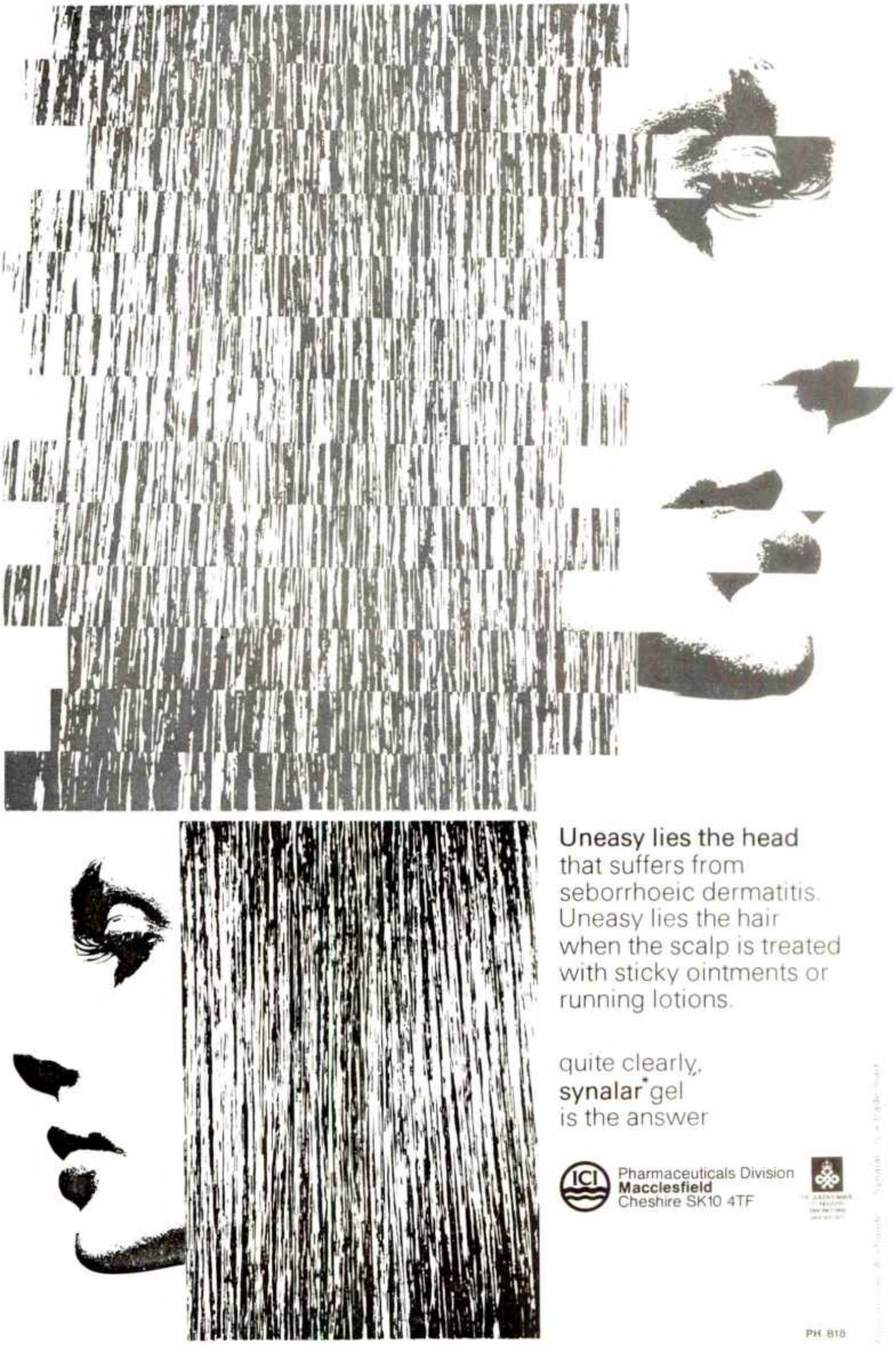

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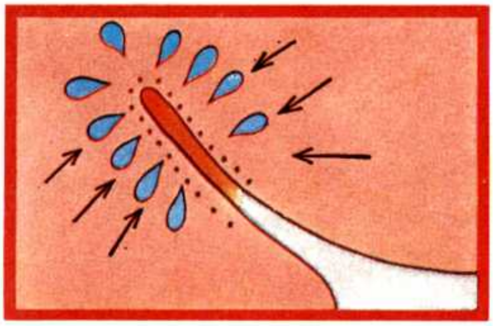
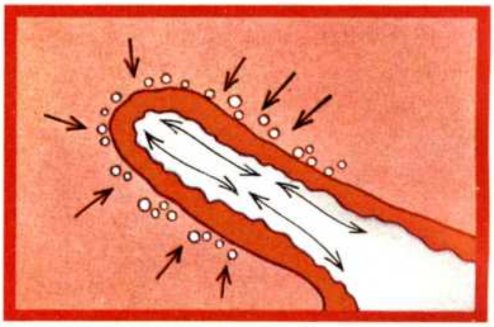
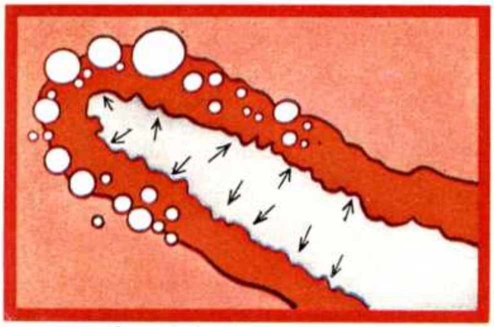


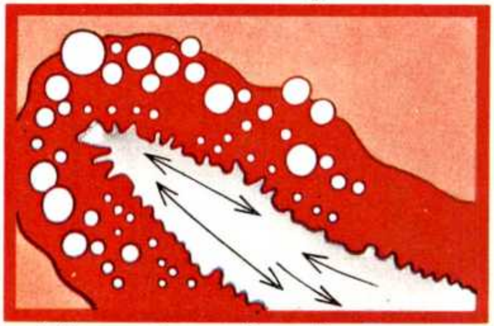
warmth and moisture



maceration and chafing



secondary infection



inflammation and pruritus

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The adverse effects of the skinfold environment militate against the success of medication in such areas.

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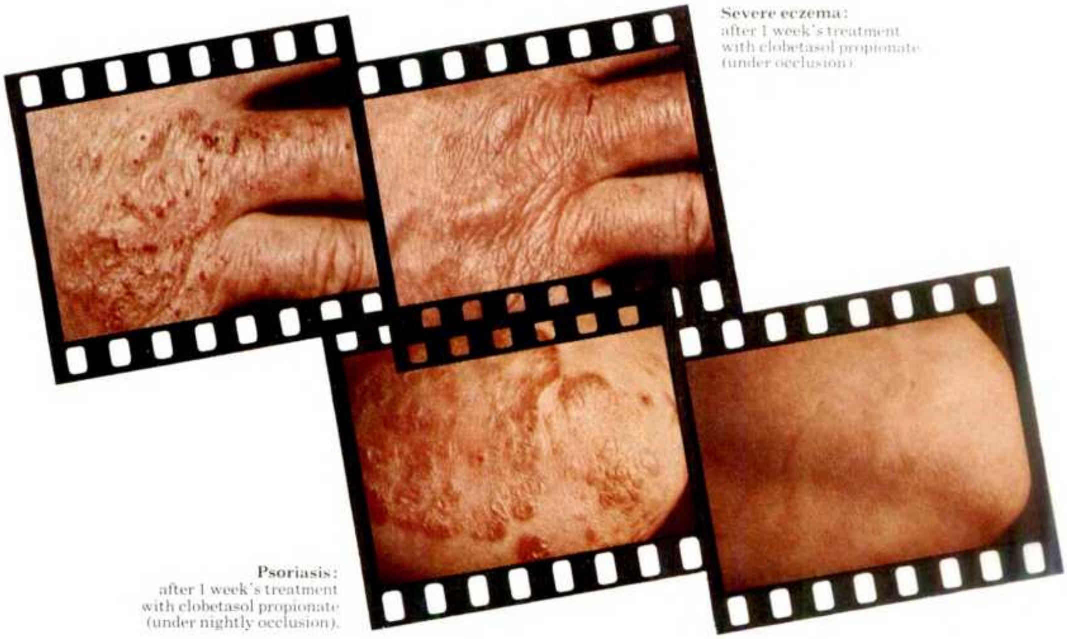

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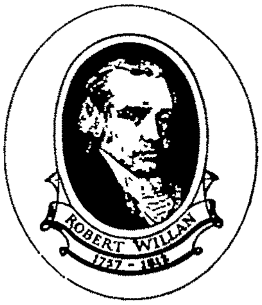

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Clinical and Laboratory Investigations

Immunological reactivity in dermatophytosis

JON M.HANIFIN, LEON F.RAY AND WALTER C.LOBITZ JR

Department of Dermatology, University of Oregon Medical School, Portland, Oregon 97201, U.S.A.

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SUMMARY

Patients with *Trichophyton mentagrophytes* and *T. rubrum* infections were compared in terms of cutaneous responses and *in vitro* lymphocyte reactivity to trichophytin and other antigens. Twelve out of fourteen *T. mentagrophytes* patients exhibited delayed cutaneous hypersensitivity to trichophytin. Most *T. rubrum* patients (thirty-seven out of forty-nine) lacked delayed reactions to trichophytin specifically, but these patients frequently manifested immediate weal reactions to this antigen. Positive lymphocyte responses correlated with the presence of delayed but not immediate cutaneous reactions. Serum from chronic *T. rubrum* patients failed to inhibit lymphocyte responses. Possible mechanisms for selective anergy in chronic *T. rubrum* patients are discussed.

Dermatologists have long been aware of acquired immunity following dermatophyte infections. Natural infections, especially with the more virulent, zoöphilic organisms, may cause secondary inflammatory lesions, such as kerions, and these patients are seldom reinfected. Numerous investigators, using the technique of experimental dermatophyte infection, have documented a gradually increasing resistance to reinfection and coincident appearance of delayed type hypersensitivity to intradermal trichophytin (Bloch & Massini, 1909; Lepper, 1969; Knight, 1972). Largely because of observations such as these, immunity has been assumed to be of the cell-mediated type.

Some patients with dermatophyte infections manifest immediate weal reactions to intradermal trichophytin. These reactions, seen more frequently in atopics and in persons infected with *Trichophyton rubrum*, can be passively transferred with serum, using the Prausnitz–Küstner technique (Marcussen, 1937). The relationship of these reactions to delayed hypersensitivity and host resistance is unclear.

In this study we examined the various cutaneous reaction patterns to trichophytin antigens in patients with *T. mentagrophytes* and *T. rubrum* infections. We also employed the technique of *in vitro* lymphocyte transformation to evaluate better the significance of cutaneous trichophytin reactivity.

MATERIALS AND METHODS

Subjects included patients with proven dermatophyte infection and normal healthy adult volunteers. Mycological identification of fungal isolates was determined by gross and microscopic morphology (Ray & Campbell, 1974), production of urease (Philpot, 1967), and, where necessary, by hair penetration (Ajello & Georg, 1957).

Antigens were prepared from *Trichophyton mentagrophytes* (TM) and *T. rubrum* (TR) by ethylene glycol extraction of bulk culture after acetone extraction (Codner *et al.*, 1961). Protein content, measured gravimetrically by the Lowry method (Lowry *et al.*, 1951), ranged between 330 and 440 μg/mg antigen. The lyophilized antigen was reconstituted in physiological saline at a concentration of 0·1 mg/ml for intradermal testing. Dermatophytin (mixed trichophytin) 1:30 and a Candida antigen (*Candida albicans*, glycero-saline extract, 1:10) were obtained from Hollister–Stier. Mumps skin test antigen (Lilly) and purified protein derivative of tuberculin (Connaught) were also used.

Skin tests were applied and read by one of two persons. Intradermal injection of 0·1 ml of test antigen was administered in all instances. Measurements of cutaneous reactions were made at 15 min and at 48 h. Immediate reactions were considered positive if the weal was ≥ 12 mm diameter or formed pseudopodia. Delayed reactions were positive if the area of induration exceeded 7 mm in diameter. Patients with negative 48 h readings were issued 'take-home' sheets on which they were instructed to record any reactions which occurred during the next 2 weeks. In the few cases in which retarded reactions occurred, these reactions were confirmed by a second test which invariably produced a 48 h response.

Lymphocytes for *in vitro* studies were isolated from 30 ml heparinized human venous blood. After sedimentation of red cells, using 3% dextran in saline, supernatant leukocytes were washed in Hank's balanced salt solution. Residual red cells were lyzed with ammonium chloride–tris buffer (Schwartz, 1971). After further washing, cells were suspended at a concentration 1×10^6 lymphocytes per ml in McCoy's 5A medium (Grand Island Biological), enriched with 15% pooled human AB serum or other sera as indicated. 1 ml aliquots of this suspension were distributed in 25 × 75 mm plastic culture tubes (Falcon). Antigens in phosphate buffered saline or saline without antigen were added to the lymphocyte suspensions. Dose–response studies showed a wide range between observable toxic effects and peak antigen stimulatory concentration. The lowest antigen concentrations causing peak stimulation were selected; TM and TR were used at a concentration of 25 μg and 50 μg per ml culture respectively. Candidin was used at a 1:100 dilution of the commercially supplied extract (Shannon *et al.*, 1966). Tubes were sealed and incubated at 37° C. After 5 days, tritiated thymidine (New England Nuclear, specific activity 2 Ci/mM, 1 μCi/ml culture) was added. 18 h later, cells were harvested and counted as previously detailed (Hanifin & Cline, 1970), after preparing cytocentrifuge smears for morphologic evaluation. Results were expressed as the average counts per minute (ct/min) incorporated into triplicate cultures, or as the ratio ct/min in antigen-stimulated culture:ct/min in saline control cultures.

RESULTS

Intradermal testing studies

Comparison of trichophytin antigens. Reactions to the three antigens, TM, TR and Dermatophytin, were compared in forty-nine infected patients as shown in Table 1. A greater proportion of patients demonstrated delayed reactivity to the TM antigen ($P > 0{\cdot}05$, $< 0{\cdot}10$ by conditional test). The frequency of immediate reactions was similar for the three antigens. Twenty-six of the forty-nine patients had identical reactions to all three antigens.

We failed to find a correlation between the infecting species and the antigen type giving positive skin reactions (Table 2). In most instances, patients reacted to both TM and TR regardless of whether they were infected with *T. mentagrophytes* or *T. rubrum*. Four patients with *T. rubrum* infections had delayed reactions to TM, but not to TR.

Correlation between infecting species and skin test reactivity. A high proportion of patients infected with *T. mentagrophytes* exhibited delayed cutaneous reactivity to one or more trichophyton antigens, as shown in Table 3. It should be noted that four of the patients with delayed reactivity were negative 48 h after the first intradermal test. Reactivity in these patients was retarded for a period of from 5 to 14 days, but in every instance retesting with trichophytin produced a weak but definite 48 h delayed reaction. No patients with *T. mentagrophytes* infection manifested an exclusively immediate response.

TABLE 1. Comparison study of reactivity to intradermal *Trichophyton mentagrophytes* (TM), *Trichophyton rubrum* (TR) and Dermatophytin antigens in forty-nine patients with dermatophytoses

	TM	TR	Dermatophytin
Immediate	27 (57%)	26 (53%)	24 (49%)
Delayed	13 (27%)	8 (6%)	8 (16%)

TABLE 2. Comparison between antigen and infecting species in patients tested with both TM and TR

Culture	No. of patients	Reactive to:			
		TR only	TM only	Both	Neither
Delayed reactions					
T. rubrum	42	0	4	3	35
T. mentagrophytes	8	0	2	4	2
Immediate reactions					
T. rubrum	42	5	3	18	16
T. mentagrophytes	8	0	0	0	8

TABLE 3. Correlation of infecting species with cutaneous reactions to trichophyton antigens

	No reaction	Immediate only	Delayed only	Immediate and delayed
49 *T. rubrum* patients	14 (29%)	23 (47%)	6 (12%)	6 (12%)
14 *T. mentagrophytes* patients	2 (14%)	0	11 (79%)	1 (7%)
21 control subjects	16 (76%)	3 (14%)	2 (10%)	0

In striking contrast, the patients infected with *T. rubrum* had a very low frequency of delayed reactions and a high frequency of immediate or absent reactivity. Thirty-seven of these forty-nine infected patients failed to express delayed reactivity to trichophytin. A number were tested as many as four times without eliciting a delayed response. These patients were otherwise healthy, with no evidence of an immunological deficiency or of diabetes mellitus. Approximately half the chronic *T. rubrum* patients with negative delayed hypersensitivity had evidence of atopy based on physical findings, past history or family history. Twenty-one of the thirty-seven manifested delayed reactivity to antigens other than trichophytin (candidin, mumps, tuberculin).

Fifty-nine percent of the *T. rubrum* patients exhibited immediate responses to trichophytin. We questioned whether a factor causing the immediate weal reactions could be inhibiting delayed responses. In an effort to examine the possibilities of local interference, we used intradermal chlorpheniramine (Chlortrimeton, 10 mg/ml, Schering) to depress immediate weal reactions, following the technique of Brostoff & Roitt (1969). This manoeuvre failed to uncover any tendency to develop delayed responses in three patients so tested.

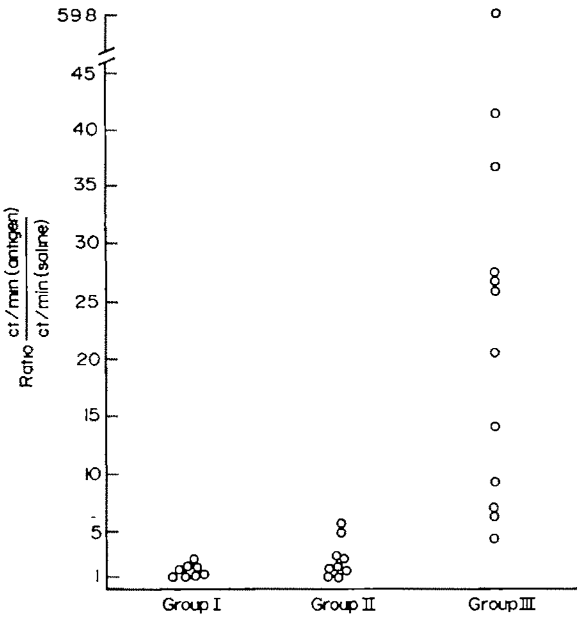

FIGURE 1. Lymphocyte incorporation of tritiated thymidine in response to *T. mentagrophytes* antigen. See text for explanation of group designations.

Lymphocyte transformation studies

As a means of further examining the cellular immune responses of infected patients, we evaluated the *in vitro* transformation of their lymphocytes in response to trichophytin and other antigens. Three groups of subjects were evaluated.

Group I. Nine controls who were normal, non-infected persons with no cutaneous reactivity to trichophytin.

Group II. Nine patients with chronic *T. rubrum* infections who lacked delayed reactivity but manifested immediate weal reactions to trichophytin.

Group III. Twelve patients with either *T. mentagrophytes* or *T. rubrum* infections who gave delayed responses to intradermal trichophytin. Some of these patients also had immediate reactions.

The results are shown in Fig. 1. Lymphocyte responses of Group III patients invariably confirmed 48 h positive skin test reactions to trichophytin. Ratios varied widely and did not consistently correlate with the magnitude of the cutaneous reaction. However, studies on two of the patients with a retarded form of delayed reactivity (i.e. whose skin test reactions appeared at 5 and 7 days) revealed low thymidine uptake ratios, not significantly different from control values.

Thymidine uptake ratios in Group II averaged 2·46 (SEM±2·56) and were not significantly increased above control ratios (1·33±0·19). Hence, the presence of immediate reactivity was not reflected by lymphocyte reactivity to trichophytin.

Candidin was used as a positive antigenic control in all lymphocyte transformation studies. Lymphocytes invariably showed significant responses to this antigen, even when unresponsive to trichophytin.

We questioned whether a factor in the serum of these patients chronically infected with *T. rubrum* might be interfering with their cell-mediated responses to trichophytin. Serum from these patients was used, in parallel with the usual AB serum, for cultures of autologous lymphocytes and trichophytin sensitive lymphocytes. We have observed no inhibition by this serum of lymphocyte responses to trichophytin or candidin.

The majority of our studies used TM to test lymphocyte responses. We questioned whether lymphocytes from patients with chronic *T. rubrum* infections might be responsive specifically to TR antigen, but in no instance did this antigen produce significantly different stimulation ratios from cultures incubated with TM.

DISCUSSION

Investigation of immunological responses to dermatophyte infections has been vigorously pursued for over 60 years, beginning with the studies of Bloch & Massini (1909). Most reports, based on techniques of experimental infection, have shown agreement on certain findings: (a) during infection, the host acquires gradually increasing resistance to reinfection; (b) sites of reinfection in immune animals closely resemble the original infectious lesion, but resolve more rapidly and the fungus is difficult to demonstrate; (c) the acquired immunity crosses species lines (i.e. infection with *T. mentagrophytes* produces immunity also to *T. rubrum* and other dermatophytes); (d) delayed type reactivity to trichophytin appears at approximately the same time as does resistance to reinfection.

Intradermal testing with trichophytin has provided another procedure for evaluating immunological responses in patients with dermatophyte infections. With the development of methods for producing large quantities of non-toxic trichophyton antigens, we have been able to combine intradermal testing procedures with *in vitro* techniques.

We have shown that our purified antigens are more sensitive than commercial materials and that there is no firm relationship between infecting species and the species from which the antigen is extracted. As in numerous other studies, the proportion of our dermatophytosis patients exhibiting delayed cutaneous reactivity to trichophytin was low, reaffirming the diagnostic limitations of this procedure.

Individuals tested with trichophytin show one of four patterns of cutaneous reactivity: (1) no reactivity; (2) immediate reactivity only; (3) delayed reactivity only; (4) both immediate and delayed reactivity. Correlating these reactions with mycological isolates from the patients allowed us to make two relatively consistent generalizations: (a) patients infected with *T. rubrum* are more likely to have either no reactivity or only immediate reactivity (74% probability); while (b) *T. mentagrophytes* patients are more likely to have delayed reactivity with or without immediate reactivity (85% probability). These differences are highly significant ($P<0\cdot005$ by chi square method). Examination of the relationships between the infecting species and the cutaneous reaction to trichophyton antigen suggested a number of approaches to a better understanding of dermatophyte immunity.

Patients infected with *T. rubrum*, often chronically over a period of many years, seldom manifested delayed skin test responses to trichophytin. We questioned whether intradermal testing was simply insufficiently sensitive, so we tested *in vitro* lymphocyte responses to trichophytin in these patients and again found no reactivity. When these patients were tested with other antigens, both *in vivo* and *in vitro*, we found that they could react and, hence, apparently had a trichophytin-specific absence of cell-mediated response. A similar selective anergy to dermatophyte infections was the subject of a case report by Marmor & Barnett (1968) describing a patient with widespread *E. floccossum* infection.

Another characteristic of *T. rubrum* patients is their high proportion of immediate reactions to intradermal trichophytin. Most evidence indicates that immediate weal reactions in the skin result from the interaction between antigen and antibodies of the IgE type. We questioned whether anti-trichophytin antibodies or other humoral factors might be responsible for the absence of cell-mediated immune responses in these chronic *T. rubrum* patients. Such a mechanism has been suggested by the finding that *in vitro* responses may be suppressed by serum from patients with breast carcinoma (Whittaker, Rees & Clark, 1971) and from a patient with mucocutaneous candidiasis (Paterson *et al.*, 1971). To date we have been unable to demonstrate any such suppression by serum from these 'anergic' *T. rubrum* patients.

Other possible mechanisms for this inability to express cell-mediated responses to trichophyton antigens must be considered. Loss of delayed hypersensitivity has been induced in animals by 'flooding' with antigenic material (Turk, 1965; Dwyer & Kantor, 1973). Also, it has been previously observed that repeated injections of trichophytin in patients with delayed reactivity will occasionally result in suppression of that delayed response and the evolution of immediate reactivity only (Jillson & Huppert, 1949). The continued presence of infecting dermatophytes may be responsible for a naturally occurring tolerant state based on similar mechanisms.

Another possible explanation for this symbiosis between human hosts and *T. rubrum* is a genetic inability of certain individuals to respond to the specific antigenic components of the organism. Thus, the organism would be rejected by all except patients who lack a population of cells capable of reacting specifically to it. There is no evidence in our chronic *T. rubrum* patients for a familial predisposition to dermatophyte infections. Whatever the mechanism, these patients appear to represent a potentially important natural model of tolerance and inadequate host resistance to a specific infecting organism.

T. mentagrophytes patients contrasted sharply with the above group in terms of cutaneous and lymphocyte responses. Twelve of the fourteen *T. mentagrophytes* patients expressed delayed skin test reactivity and, in each case tested, 48 h responses were confirmed by lymphocyte reactivity to trichophytin. The retarded delayed reactors showed low levels of lymphocyte reactivity, suggesting that they had a lesser degree of cell-mediated responsiveness to trichophytin. The apparent 'booster' effect of intradermal trichophytin, causing more rapid development of delayed responses on retesting these patients, has also been noted by Jones (1972). We found no evidence to support a previous suggestion (Wood & Cruikshank, 1962) that patients with trichophytids tended to have retarded-type reactions. Delayed hypersensitivity to trichophytin has been widely considered to be an indicator of resistance to infection, and this would be generally consistent with the more acute, short-lived nature of the infection in *T. mentagrophytes* patients. Our studies have, however, revealed a few patients with strong cell-mediated responses who were unable to reject their chronically infecting dermatophytes. Such patients are described in another report (Hanifin & Ray, 1973) and suggest that delayed reactivity is not in itself resistance against dermatophyte infection.

This report represents the preliminary observations in a continuing study of human immunological responses to dermatophyte infections. The mechanisms of these responses are far from clear, but such patients provide a fascinating, abundant population of immunologically abnormal reactors.

The study of such patients, using more refined antigens coupled with newer immunological techniques, offers an unusual opportunity to investigate basic immunological mechanisms in otherwise healthy individuals.

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British Journal of Dermatology (1974) **90**, 9.

Improved fluorescence staining technique for estimating turnover of the human stratum corneum

L.H.JANSEN,* M.T.HOJYO-TOMOKO AND A.M.KLIGMAN

State University of Utrecht, Institute of Dermatology, Utrecht, The Netherlands,* and Department of Dermatology, University of Pennsylvania School of Medicine, Duhring Laboratories, Philadelphia, Pennsylvania 19104, U.S.A.

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SUMMARY

The replacement time of the human stratum corneum of fifteen body regions was determined by measuring the days required for a strongly substantive fluorescent marker, Dansyl chloride, to disappear. The horny layer was stained by a 24-h patch exposure to 5% Dansyl chloride (5-dimethyl-amino-1-naphthalene-sulphonyl chloride) in petrolatum.

In most body regions the renewal time for the horny layer was about 2 weeks, in agreement with the findings of other investigators.

The advantage of Dansyl chloride over another compound, tetrachlorosalicylanilide, which has been used for this purpose, is that it is not an irritant.

Counting mitoses or labelling the nucleus or cytoplasm with radioactive substances are the methods which researchers use to estimate the proliferative activity of integumentary epithelia. Clinicians must have simpler methods, even if these are less precise.

The time required for a substantive dye to disappear from the fully stained horny layer provides an estimate of its replacement time. The transit of cells through the horny layer is of course a function of the rate of new cell production by dividing cells. The stratum corneum replacement time is thus an indirect measure of mitotic activity. This principle was reduced to practice by Baker & Kligman (1967), who utilized tetrachlorosalicylanilide (TCSA) to stain the horny layer. While the figures for horny layer renewal corresponded to accepted values (about 2 weeks for the trunk) TCSA had two serious limitations. It is a potent contact sensitizer; moreover, about a third of the tests have to be discarded because of irritation. Inflammation accelerates cell production and the dye then disappears in an abnormally short time.

We have come across another highly substantive fluorescent marker which is completely non-irritating and which rarely sensitizes. Dansyl chloride, 5-dimethyl-amino-1-naphthalene-sulphonyl chloride, combines avidly with amino groups and thus is useful for fluorescent tagging of proteins. It is a highly stable substance with such extreme resistance to acids that it is widely employed for NH_2-terminal analyses of proteins and peptides. It is insoluble in most ordinary solvents. We origin-

Reprint requests to Dr Kligman, Duhring Laboratories, 3600 Spruce Street, Philadelphia, Pennsylvania 19104, U.S.A.

ally used a 1% solution in equal parts of ethanol and acetone applied to the skin for 2 h under an occlusive patch. A precipitate formed in a day or so, necessitating the use of fresh solutions. After considerable trial and error we finally found it advantageous to use petrolatum as a vehicle.

METHOD

Dansyl chloride was obtained from the Mann Research Labs., New York City. It was finely triturated into white petrolatum at 5% by weight. The ointment was liberally smeared over a 2.5 cm square of cotton felt (Webril, Curity) and this was occlusively fastened to the skin under overlapping strips of impermeable plastic tape. Complete penetration generally occurred by 24 h, as judged by adhesive tape stripping the glistening layer. When complete, the surface still fluoresced lightly even when no more cells would adhere to the tape. We found that penetration within 24 h could be guaranteed if the patch was renewed after 6 to 8 h. Actually a new patch need not be put on. It is sufficient to lift the felt, rub the site vigorously with the finger and refasten the patch. This evidently redistributes the highly insoluble fluorescent marker and effects greater contact at the surface.

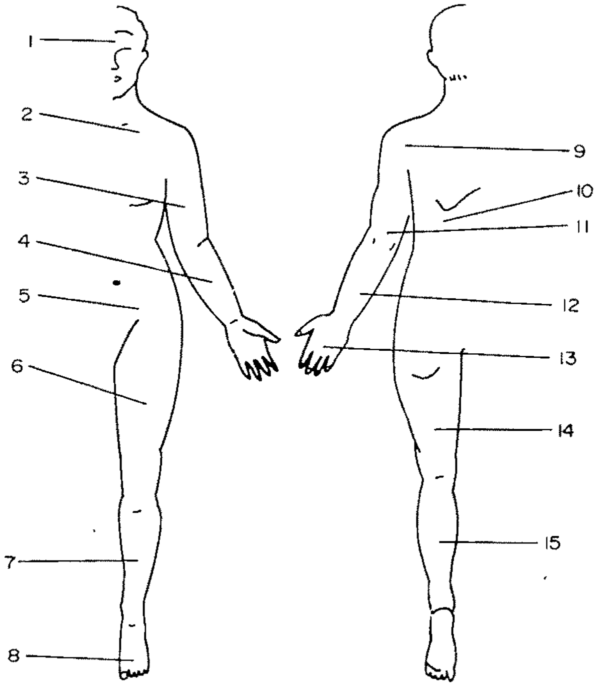

FIGURE 1. Sites selected for the measurements.

The end point was the number of elapsed days till disappearance of fluorescence by Wood's light examination.

RESULTS

Replacement times were determined for fifteen sites in twelve adult white males and females. The regions studied are depicted in Fig. 1 and the results are given in Table 1.

Except for the forehead (8·5 days), the dye disappeared in about 2 weeks. This accords with values secured by others using different methods (Weinstein & Van Scott, 1965; Rothberg, Crounse & Lee, 1961). It is also well established that turnover is faster on the head (Plewig, Fulton & Kligman, 1971). Generally, the times were somewhat longer for the lower than for the upper extremities. There were, of course, individual differences.

Regional comparisons are valid only when the number of cell layers is the same. When it is not, it is useful to convert replacement time to rate, as Baker & Blair (1968) have done. This is done by dividing the time in days or hours by the number of cell layers. Obviously, if cell generation is the same, the time for a cornified cell to reach the surface will be greater when there are more cell layers. As it turns out, the horny layer in most regions is 15–20 cell layers thick. For 15 layers the replacement time would be 14/15 = 0·93, or a little less than one day per cell layer. For 20 cell layers, the value would become 0·7.

TABLE 1. Replacement time in days

Site	Subjects												Average
	1	2	3	4	5	6	7	8	9	10	11	12	
1	12	7	6	7	12	8	8	6	9	9	8	11	8·5 ± 0·61
2	9	18	8	10	12	15	11	13	13	13	12	18	12·6 ± 0·91
3	12	14	8	16	14	17	12	11	14	13	14	15	13·3 ± 0·69
4	10	14	8	10	14	15	8	11	15	—	14	15	17·2 ± 0·98
5	11	12	8	12	12	9	9	8	9	8	17	23	11·0 ± 1·29
6	11	18	11	10	14	15	8	13	13	13	21	21	14·0 ± 1·19
7	16	18	11	10	16	17	12	11	13	13	21	23	15·1 ± 1·20
8	19	18	15	10	14	15	12	16	13	17	16	21	15·5 ± 0·88
9	12	14	11	10	12	15	8	11	13	13	12	13	12·0 ± 0·54
10	15	13	11	10	11	15	12	15	13	15	16	29	14·5 ± 1·43
11	15	14	11	10	10	15	12	11	13	15	12	27	13 7 ± 1·32
12	12	14	10	9	13	—	8	12	11	—	16	14	11·9 ± 0·65
13	9	12	11	10	14	14	12	13	12	13	14	18	12·6 ± 0·67
14	15	18	13	12	14	17	8	13	16	13	16	23	14·8 ± 1·06
15	26	18	13	10	18	15	12	13	14	13	21	—	15·7 ± 1·25

DISCUSSION

Dansyl chloride is a highly substantive fluorescent marker which binds avidly to proteins. The disappearance time was not shortened by soap and water washing for 1 min twice daily. The sites may be left uncovered and are invisible in daylight.

We conducted some elementary tests to evaluate substantivity. Discs of horny layer were secured by the cantharidin blister technique from skin treated as above with 5% Dansyl chloride for 24 h. Boiling these discs for 2 h in a water bath extracted very little of the dye, indicating that water soluble nitrogenous components are not important in binding. Extraction of the discs with chloroform methanol (2:1) for 12 h at room temperature still left the discs brightly fluorescent, though more of the dye was in solution. Evidently, lipids play no role in attracting the dye.

When undyed discs of horny layer were first boiled for 2 h to extract water soluble materials, staining in 1% Dansyl chloride in chloroform was just as intense as with normal horny layer. All these observations strongly point to binding of the marker to the insoluble fibrous proteins which make up the backbone of keratin.

In contrast to TCSA, no instance of contact sensitization occurred in these studies. Yet it would be surprising if a sulphonamide type of compound, so highly reactive with proteins, was without contact sensitizing potentialities. This in fact turned out to be the case. Four of twenty-three subjects became sensitized by the maximization procedure for assaying contact allergens in man (Kligman, 1966). By the same technique virtually all subjects became sensitized to TCSA. That test, however, involves repeated intense exposure of chemically damaged skin. The risk of sensitization by a 24 h exposure of normal skin is quite small.

Mild phototoxicity was demonstrated when treated skin was strongly irradiated with long wavelength ultra-violet light. Many fluorescent compounds have this property. Sun exposure did not produce any abnormal responses in our test subjects.

Recently, Halprin (1972) has questioned whether 28 days, a widely quoted figure, is truly the turnover time of the entire epidermis. He argued persuasively that the time may actually be nearly twice as great. His calculations, however, do not apply to the value for horny layer replacement. The figure of 2 weeks has been confirmed once more in this study. Baker & Blair (1968) found that the time was lengthened in old age. We too showed that after about the age of 70, the dye took 20–33 days to disappear from the skin of the lower legs.

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Civatte bodies and the actively dividing epithelial cells in oral lichen planus

N.G.EL-LABBAN AND IVOR R.H.KRAMER

Department of Pathology, Institute of Dental Surgery, University of London, and
Eastman Dental Hospital, Gray's Inn Road, London WC1X 8LD

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SUMMARY

The relationship between Civatte bodies and epithelial cells in mitosis was investigated in lichen planus lesions of the oral mucosa. The nuclear material in Civatte bodies was often very similar to that in normal dividing basal epithelial cells. In addition, cells were found with clearly identifiable chromosomes, but with cytoplasmic changes of Civatte bodies. These results suggest that Civatte bodies are probably the outcome of degenerative changes affecting epithelial cells in various stages of mitosis.

In a previous report on Civatte bodies in oral lichen planus (El-Labban, 1970,) we showed that the eosinophilic masses commonly referred to as colloid or Civatte bodies, seen in the deeper layers of the epithelium and also in the immediately subjacent connective tissue, originate from epithelial cells that undergo certain degenerative and keratinizing changes. However, in that report it was not clearly shown which of the epithelial cells were more prone to the degenerative changes.

The purpose of this paper is to discuss the possible relationship between these bodies and actively dividing epthelial cells.

MATERIAL AND METHODS

The material consisted of twenty-two specimens taken from lesions of the buccal mucosa diagnosed histologically as lichen planus. The material for electron microscopy was fixed in osmium tetroxide buffered with veronal acetate, or fixed in cacodylate-buffered glutaraldehyde and post-fixed in buffered osmium tetroxide. After embedding in Araldite, ultra-thin sections were cut with a glass knife on an LKB1 ultratome, stained with uranyl acetate and lead citrate and examined in a Ziess EM 9S2 electron microscope.

RESULTS

The numbers of degenerating epithelial cells varied considerably from case to case, and in different parts of the same specimen.

Nuclear material with and without a nuclear membrane, as well as small nuclear fragments, were often seen in those degenerating epithelial cells identified as Civatte bodies. They appeared in various positions in the cell, but were commonly seen at the periphery (Figs. 1, 2 and 3). They showed

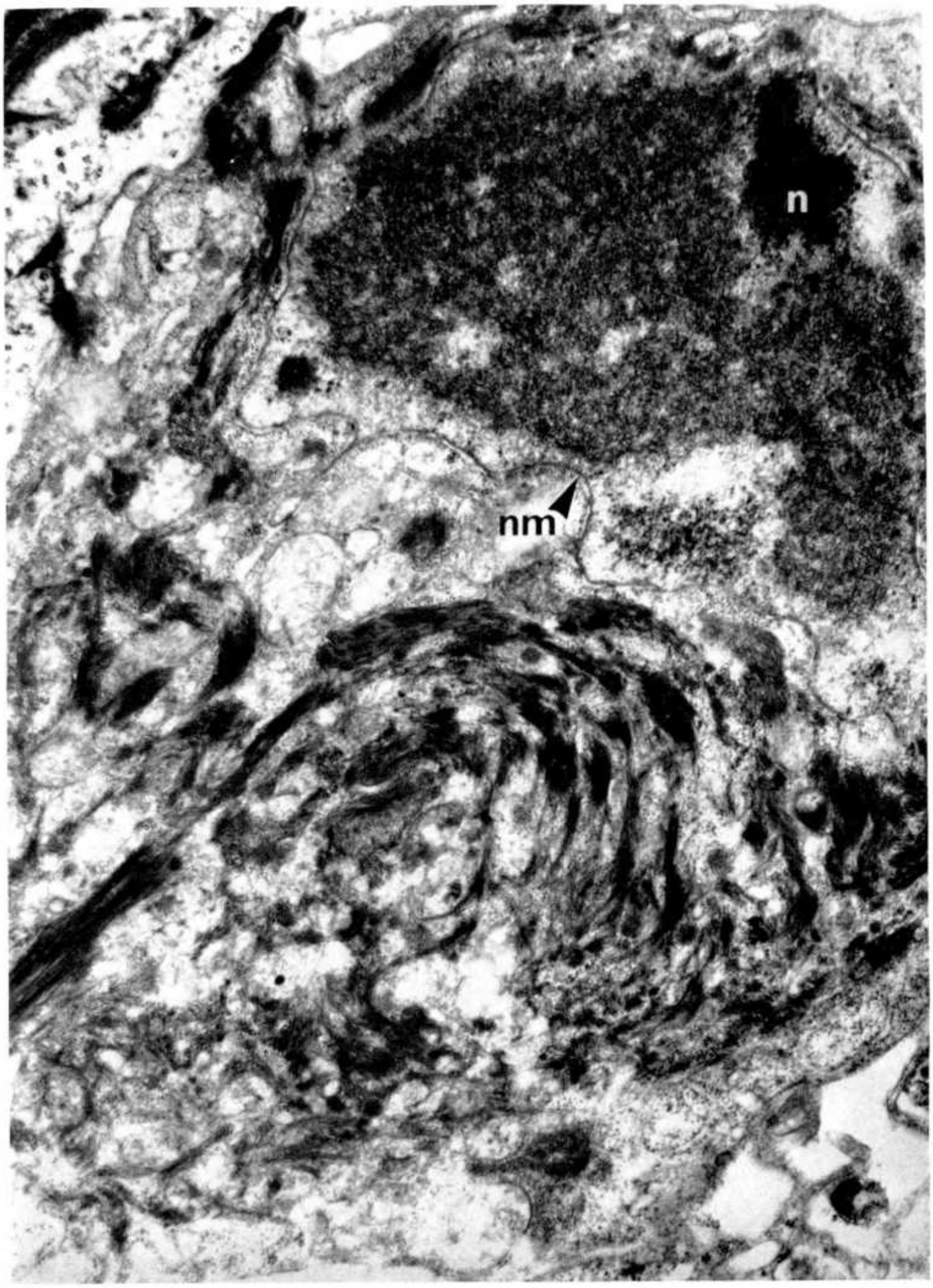

FIGURE 1. A degenerate epithelial cell in the basal layer from a lichen planus lesion, containing a large accumulation of electron dense bundles of tonofibrils characteristic of those epithelial cells forming Civatte bodies. The nucleus is kidney-shaped and peripherally situated. The chromatin granules are densely packed and the electron dense area (n) could be a nucleolus. The nuclear membrane (nm) appears to be intact. The shape of the nucleus and the arrangement of the chromatin granules resemble a nucleus in late telophase (× 18,000).

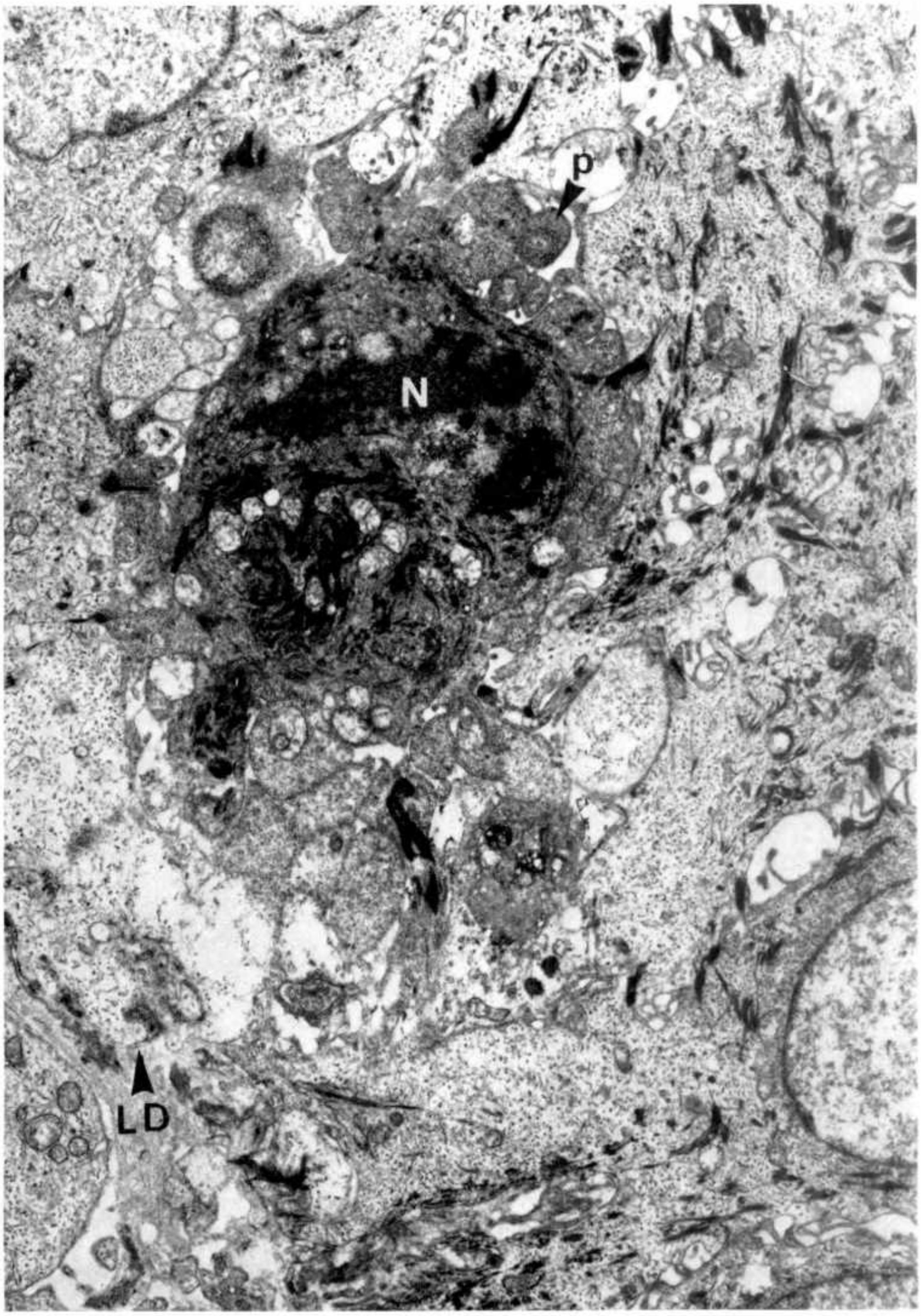

FIGURE 2. A degenerating epithelial cell in the basal layer of a lichen planus lesion showing electron dense tonofilaments and a large number of particles. Some of the particles form clusters which tend to separate from the main body of the cell (p). The plasma membrane is ill-defined in most areas. The nucleus (N) is peripherally located and contains chromatin granules in an arrangement similar to that of daughter cell nuclei. LD is the lamina densa (× 6800).

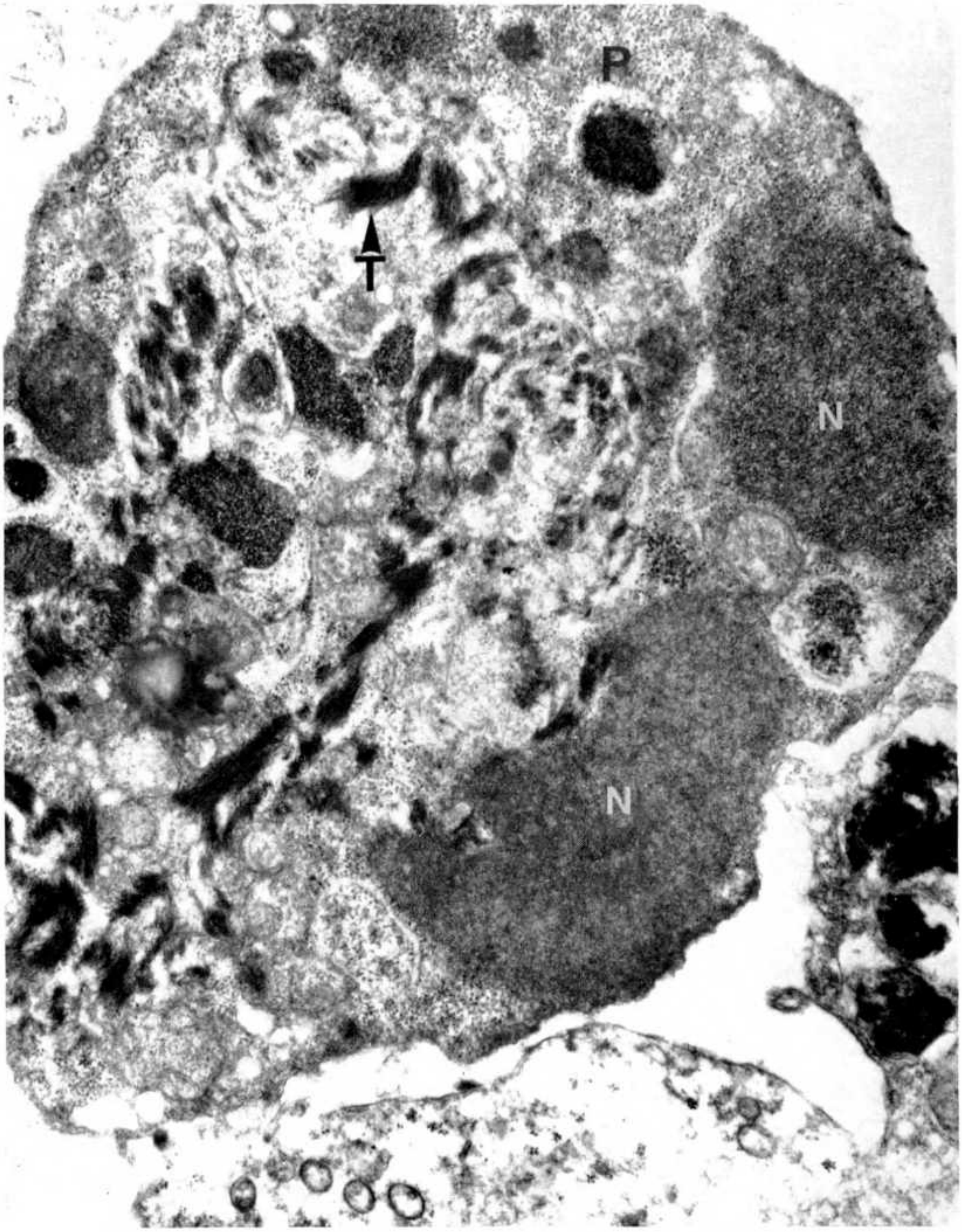

FIGURE 3. A completely isolated degenerate epithelial cell separated from the surrounding cells by a wide space. Note the electron dense tonofilaments (T), the large number of particles (P) and the ill-defined appearance to the plasma membrane. The nuclear fragments (N) show no limiting membrane and are formed of aggregations of densely packed chromatin granules (× 25,500).

different morphological appearances, not only in different cells but also in the same cell. Some of the nuclear masses were kidney-shaped, especially those appearing at the periphery and showing a nuclear membrane. Some masses of nuclear material were rod-shaped, were found mainly in the middle of the cell and showed no nuclear membrane (Fig. 4). Others appeared as fragments of various sizes, some of which had no nuclear membrane and resembled chromosomes (Fig. 5). Some of the nuclei which showed a nuclear membrane contained compact chromatin granules (Fig. 1), similar to those of nuclei in telophase, exhibiting an ill-defined envelope around the coalescent mass of chromosomes; other nuclei showed an uneven distribution of the chromatin granules (Fig. 2), similar to that of daughter cell nuclei.

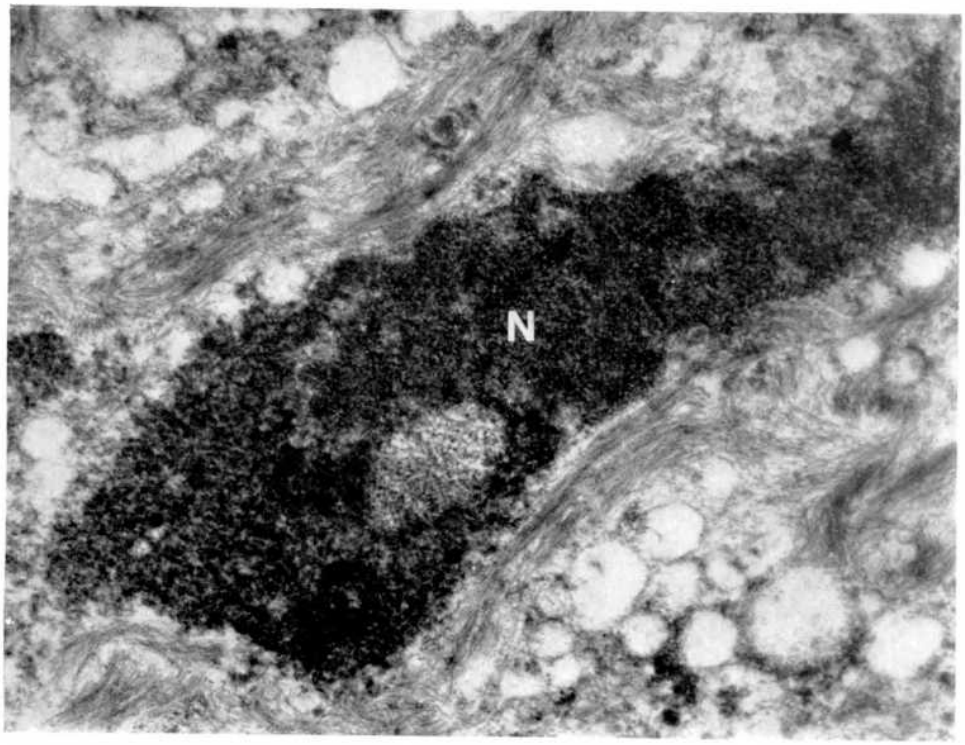

FIGURE 4. Part of a degenerate epithelial cell from a lichen planus lesion showing a centrally located nuclear mass (N) with no nuclear membrane. The cytoplasm shows a large number of tonofilaments of low electron density (× 45,000).

There were also cells containing two nuclei in the position of a recently divided nucleus, at the opposite poles of the cell (Fig. 6), and cells were found showing recognizable chromosomes, a centriole and spindle tubules located to one side of the cell (Fig. 7). With all these variations, the cytoplasm showed degenerative changes characteristic of cells that were changing into Civatte bodies, as described in detail by El-Labban (1970). In normally dividing basal epithelial cells, the tonofilaments are few, are of low electron density, and are arranged peripherally. In contrast, the cells described here contained a larger number of tonofilaments of greater electron density, and these were more randomly distributed in the cytoplasm. The variation was not confined to the tonofilaments; ribosomes and mitochondria were also much more numerous than in cells undergoing normal mitosis. In normally dividing cells the desmosomes are few, but the plasma membrane shows no defects, whereas in these cells the plasma membrane always showed areas that were ill-defined (Figs. 2 and 3), and membrane-bound clusters of particles were often separated from the cells at the periphery (Fig. 2). These particles measured approximately 15 nm and resembled ribosomes. Electron dense particles measuring about 30–50 nm in diameter were often seen in the nuclei found in Civatte bodies, especially those showing a nuclear membrane (Fig. 8). They appeared to have an inverse relationship to the amount of demonstrable chromatin in the nucleus and were sometimes aggregated close to nucleoli.

The 'nuclear bodies' seen in the epithelial cells in lichen planus, and apparently attributable to infolding of the nuclear membrane (El-Labban & Kramer, 1972) were rarely seen in the nuclear material within the Civatte bodies.

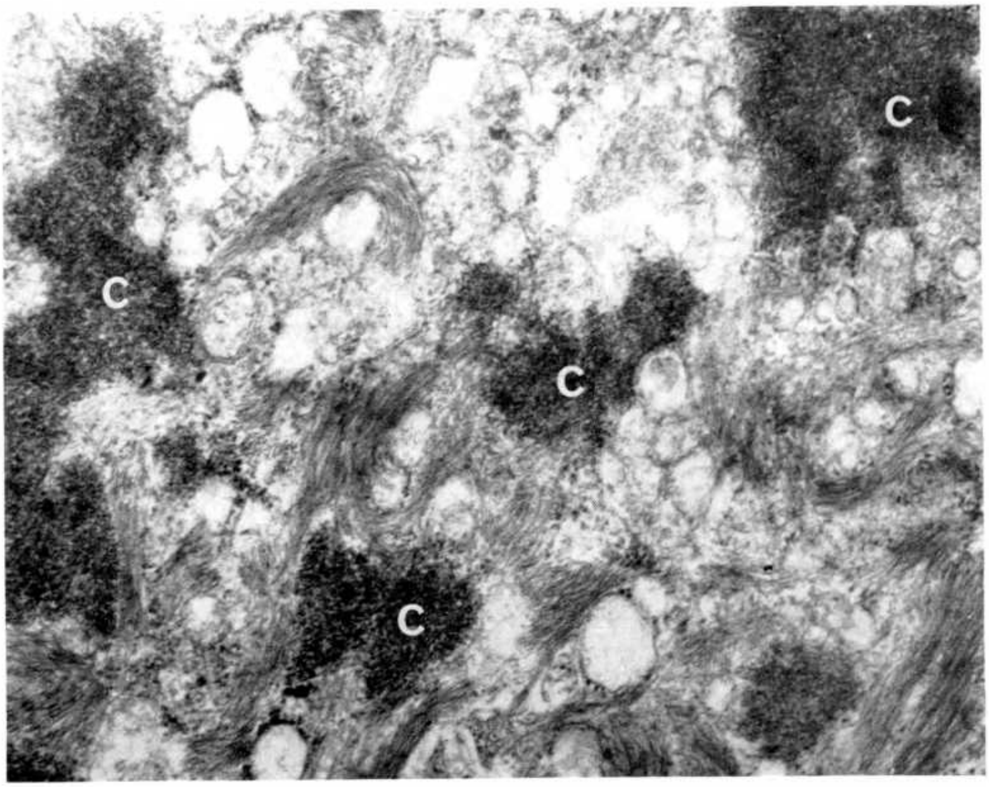

FIGURE 5. Part of a degenerate epithelial cell from a lichen planus lesion showing the accumulation of a large number of bundles of tonofibrils of low electron density, characteristic of Civatte bodies. The nuclear fragments (C) exhibit no limiting membrane and resemble chromosomes (× 34,000).

DISCUSSION

Nuclear material has often been described in Civatte bodies, using either the light microscope alone or both light and electron microscopy (Sabouraud, 1910; Gougerot & Civatte, 1953; Thyresson & Moberger, 1957; Andreasen, 1968; El-Labban, 1970). However, Ebner & Gebhart (1972) failed to observe rudiments of nuclei in these bodies. In the present study it is shown that the cytoplasmic changes are indicative mainly of degeneration and keratinization, and it is suggested that the appearances of most of the nuclei and nuclear fragments seen in these bodies indicate the involvement of an actively dividing cell.

If a keratinocyte were to be affected by a change towards keratinization during the process of mitosis, then the cytoplasmic changes of keratinization would appear concurrently with nuclear and other changes characteristic of mitosis. The cytoplasmic changes that lead to the development of Civatte bodies, and the evidence that these changes are indicative of keratinization, have already been discussed in a previous paper (El-Labban, 1970). The nuclear material found in many of these bodies was often in the form of multiple small aggregations giving a strongly positive Feulgen reaction (El-Labban, 1972).

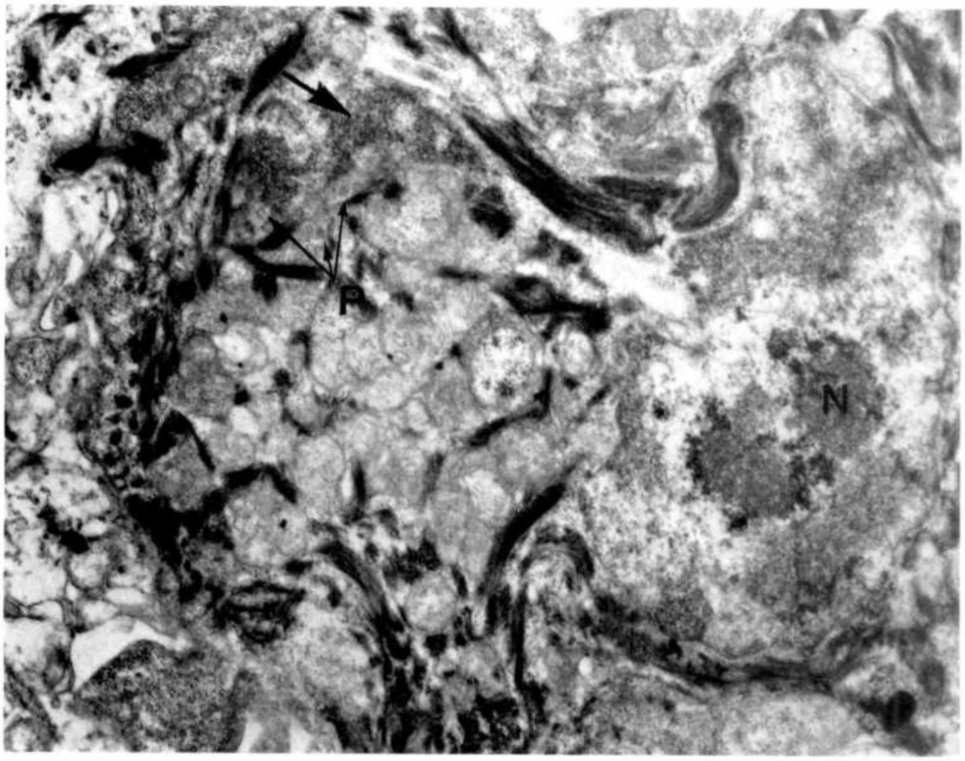

FIGURE 6. An epithelial cell from a lichen planus lesion, showing two nuclei at its opposite poles. One of the nuclei (N) is clearly identified and shows uneven distribution of the chromatin granules characteristic of daughter cell nuclei. The other (arrow) is tangentially cut and shows many nuclear pores (P). The cytoplasmic changes are clearly those of degeneration; large numbers of tonofilaments, aggregates of degenerate mitochondria, and accumulations of particles at the periphery can be seen (× 15,000).

At the ultrastructural level, these masses often had the featureless appearance, and the absence of a nuclear membrane, that are typical of chromosomes (Figs. 3, 4 and 5); furthermore, some of the cells affected clearly showed chromosomes, centrioles and spindle tubules as shown in Fig. 7. These findings, together with the presence of degenerating cells containing two nuclei, corresponding in site and morphology to the divided daughter nuclei in normal mitosis, strongly suggest that

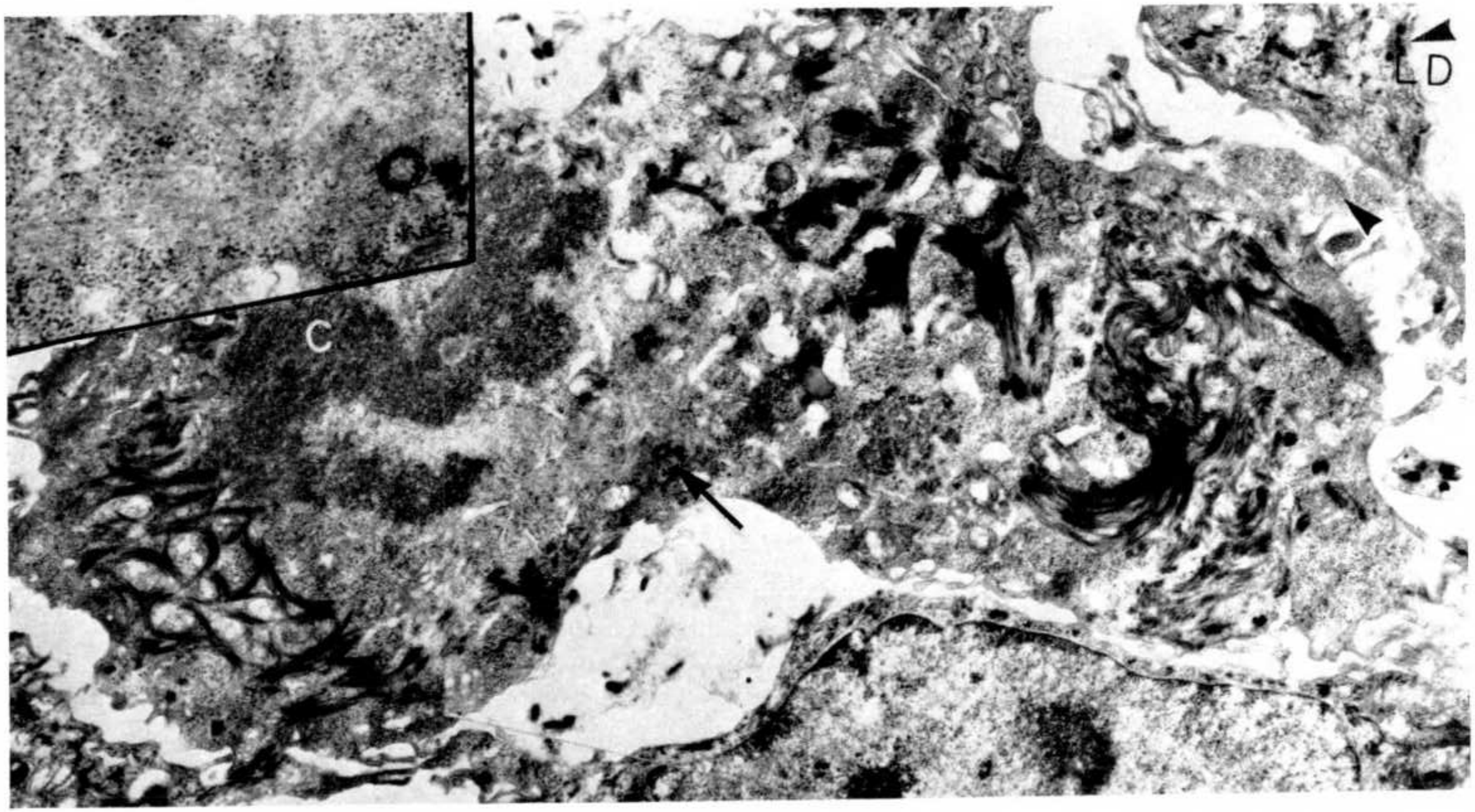

FIGURE 7. A composite electron micrograph of an epithelial cell from a lichen planus lesion containing a large number of electron dense bundles of tonofilaments and an accumulation of particles at the periphery tending to separate from the cell (arrowhead at right of micrograph). These are features similar to those seen in the degenerate epithelial cells forming Civatte bodies. However, the cell contains chromosomes (C) a centriole (arrow) and spindle tubules which are characteristic features of cells undergoing mitosis. The inset is a high power view of the centriole and some of the spindle tubules. LD is the lamina densa (× 9500 and 48,000 respectively).

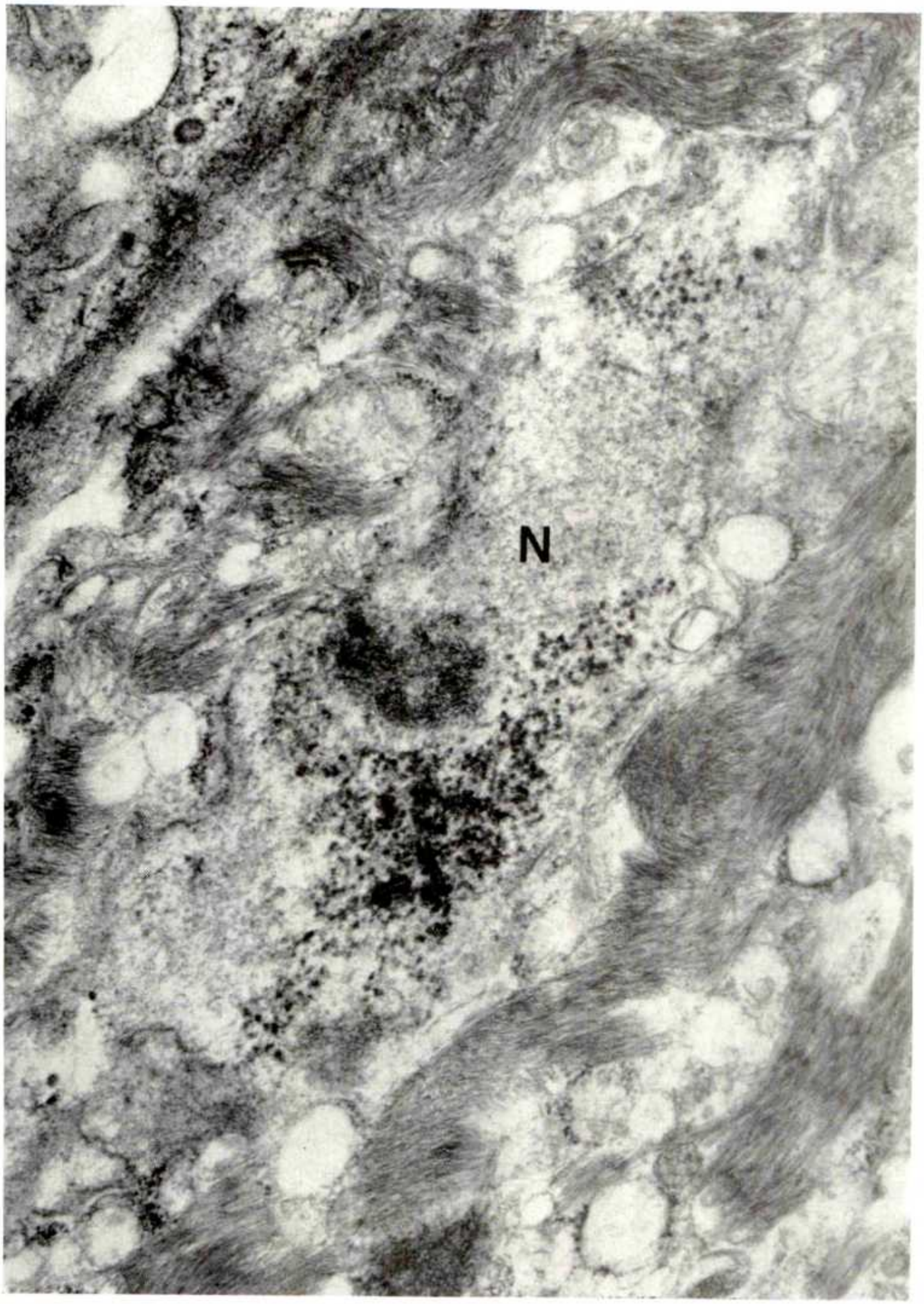

FIGURE 8. Large number of electron dense particles appearing in the nucleus (N) of one of the degenerating epithelial cells (Civatte body) from a lichen planus lesion (× 42,500).

P, 25,462

the cells from which Civatte bodies are derived are those engaged in mitosis. The variation in morphological features of the nuclear material appearing in these cells suggests that the epithelial cells can be affected at any stage during division. However, with the advance of the degenerative changes in these cells the recognizable features of mitosis can no longer be demonstrated. The electron dense particles measuring 30–50 nm appearing within the nuclei in Civatte bodies (Fig. 8) are morphologically similar to particles seen in nuclei in the surface keratinized layer of lichen planus lesions (El-Labban, 1972). This might suggest a non-specific change, perhaps of a degenerative nature. However, these particles do bear a resemblance to virions seen in wart virus infected skin (Lutzner, 1967). Further investigations are necessary to identify their nature and significance in these degenerating cells.

The finding of epithelial cells showing the features both of mitosis and of keratinization has also been shown in Bowen's disease by Seiji & Mizuno (1969). However, the factors involved in the production of such cells and in the tissue responses to their presence are probably different.

In their description of the 'dyskeratotic cells' in Bowen's disease, Seiji & Mizuno stated that these cells were characterized by an aggregation of tonofilaments in fascicular form and by a decrease in desmosomal tonofilament attachments. These findings are similar to the changes seen in cells undergoing Civatte body formation. However, Seiji & Mizuno made no reference to any ill-defined appearance of the plasma membrane of the affected cells, or to any accumulation of a large number of particles at the periphery and their eventual separation from the cell.

The initial changes that interfered with the mitotic division in epithelial cells and resulted in Civatte body formation were not clearly identified. However, the changes in the cytoplasm and plasma membrane appeared more severe than those affecting the nuclei, thus suggesting that changes in the nuclei may follow those in the cytoplasm and the plasma membrane.

Seiji & Mizuno stated that the displaced tonofilament fascicles become entangled with the mitotic apparatus, interfering with normal cell division. It has also been stated that during mitosis a special cytoplasmic environment, or viscosity, is required for the organization of the spindle (Swann, 1952).

In the present material, the cytoplasmic organelles involved were not only tonofilaments but also the mitochondria and ribosomes. It is difficult to determine with certainty which of these changes has the most profound effect on the mitotic apparatus. However, the changes in the plasma membrane of these cells may suggest a lytic action which might lead to changes in their internal environment, interfering with, and terminating, normal division.

The present investigation did not show the reasons for the selective involvement of cells undergoing mitosis. However, it could be that the exposure of the 'hot spots' during mitosis (Tooze, 1971) results in the formation of areas particularly susceptible to injury.

In a previous study on oral lichen planus (El-Labban, Lucas & Kramer, 1971), we were able to compare changes in lichen planus with leukoplakia and other keratotic lesions, and in a further investigation (El-Labban, 1972) it was also possible to compare lichen planus lesions with normal mucosa taken from corresponding sites. The results of the first study showed that the mitotic values were lower in lichen planus than in leukoplakia and the other keratotic lesions. However, comparing lichen planus lesions of the buccal mucosa with normal buccal mucosa, we found more epithelial cells in mitosis in lichen planus lesions than in normal mucosa. This finding probably agrees with findings of Marks, Black & Wilson Jones (1973), who showed an increased labelling index for the epidermis of the lichen planus papule. However, labelling of cells does not necessarily indicate that the cell would proceed to mitosis, as these authors also found that some of the swollen eosinophilic cells in the basal zone were labelled.

As most mitotic activity in the normal epithelium is in the basal cell layer, a particular susceptibility of dividing cells would account for the pronounced changes in this region in lichen planus.

ACKNOWLEDGMENTS

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The effects of experimental skin damage in patients with lichen planus

A PRELIMINARY COMMUNICATION

L. STANKLER AND S.W.B. EWEN

Department of Dermatology, Aberdeen Royal Infirmary, and
Department of Pathology, University of Aberdeen.

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SUMMARY

Experimental scratching induced phloxinophilic eosinophilic bodies in unaffected skin of ten patients with active lichen planus. This reaction was inhibited by concomitant local injection of methylprednisolone acetate and could not be induced in patients with inactive lichen planus, in patients with other dermatoses, nor in healthy subjects. The findings are consistent with a viral aetiology for lichen planus.

Ellis (1967) examined 100 classical lesions of lichen planus (LP) with the light microscope with haematoxylin and eosin, and noted eosinophilic bodies presenting as deeply staining intracellular material in thirty-seven sections. According to Lever (1961), these bodies are found especially in early lesions and probably result from degeneration of epidermal cells.

Although the Koebner reaction is known to occur in patients with LP, apart from the limited investigations of Depaoli (1953) there appears to be very little information on the effects of experimental skin damage in this condition. The present investigation was conducted to assess the effect of skin damage on the formation of eosinophilic bodies in patients with LP.

PATIENTS AND METHODS

Ten patients with the classical clinical features of LP, five normal subjects, and nineteen patients with various dermatoses (ten psoriasis, two sarcoidosis, two mycosis fungoides, three eczema, two acne vulgaris) took part in the investigation. Ten patients with widespread LP of less than 1 month's duration were considered 'active'. Five of these patients were again investigated as the disease resolved. All the tests were carried out on the flexor aspect of the forearm. In all subjects a cruciate scratch was made with a syringe needle so as to induce bleeding. In each of two patients with active LP, two scratches were made and 0·2 ml methylprednisolone acetate (Depo-Medrone, Upjohn) was injected into one of them. In one patient with active LP, three cruciate scratches were made and 3 mm punch biopsies taken at 6, 24 and 54 h respectively. In all other subjects 3 mm punch biopsies were taken after a period of 2 weeks. Punch biopsies were taken of three LP lesions which appeared spontaneously. All the biopsy material was fixed in neutral buffered formalin, embedded in paraffin wax, and 5 μm thick sections were ribboned and stained with haematoxylin and eosin or periodic acid-Schiff (PAS). A few sections were stained with phloxin–tartrazine (Lendrum, 1947).

RESULTS

All ten cases of active LP which had been scratched showed similar histological features at 2 weeks. In the basal zone and in the subjacent connective tissue at the site of damage, there was an intense non-specific chronic inflammatory cell infiltrate consisting of lymphocytes, histiocytes, macrophages (some of which contained melanin granules), scanty plasma cells and the capillary loops of new blood vessels (Fig. 1). The location of this infiltrate in the subepidermal connective tissue, rather than in the basal epidermis, was confirmed by PAS staining. The basement membrane was separated from the viable epidermal cells by a zone of epidermal cells which appeared either completely eosinophilic or contained an eosinophilic inclusion body causing displacement of the nucleus. If such an inclusion body had caused nuclear displacement, then the nuclear chromatin appeared condensed and pyknotic. The surrounding epidermis showed no abnormality. The eosinophilic bodies were phloxinophilic when stained by Lendrum's phloxin–tartrazine method (Fig. 2). In three patients with LP, who had partially improved, although eosinophilic bodies were induced by scratching they were less obvious

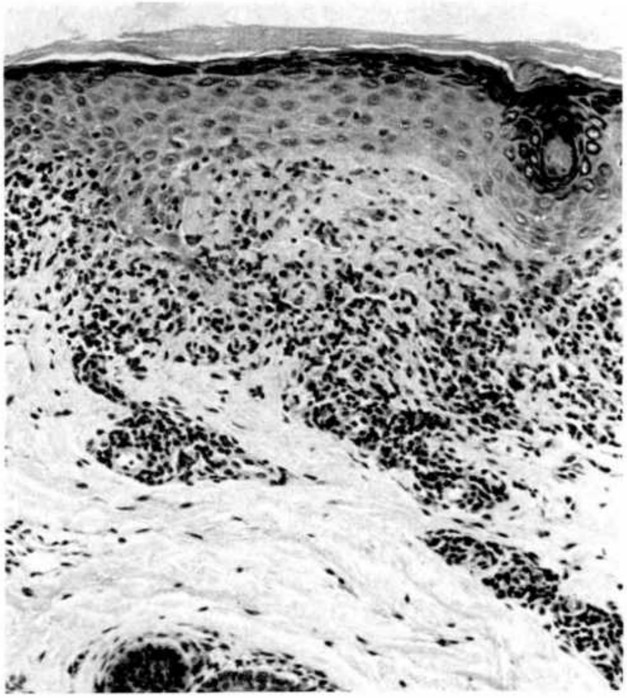

FIGURE 1. Eosinophilic bodies in lower epidermis, and non-specific chronic inflammatory dermal infiltrate, 2 weeks after scratching (H & E, × 150).

and the other histological changes described above were less marked. Attempts to induce eosinophilic bodies by scratching were unsuccessful in two patients with inactive LP. Eosinophilic bodies were not observed in biopsies taken at 6, 24 and 54 h after scratching the skin in the patient with active LP. Methylprednisolone acetate inhibited the formation of eosinophilic bodies, and the chronic inflammatory cell infiltration was absent in two patients so treated. The three spontaneous lesions were not phloxinophilic when stained with the phloxin–tartrazine stain. Scratching did not induce eosinophilic bodies in healthy subjects or in the patients with dermatoses other than LP.

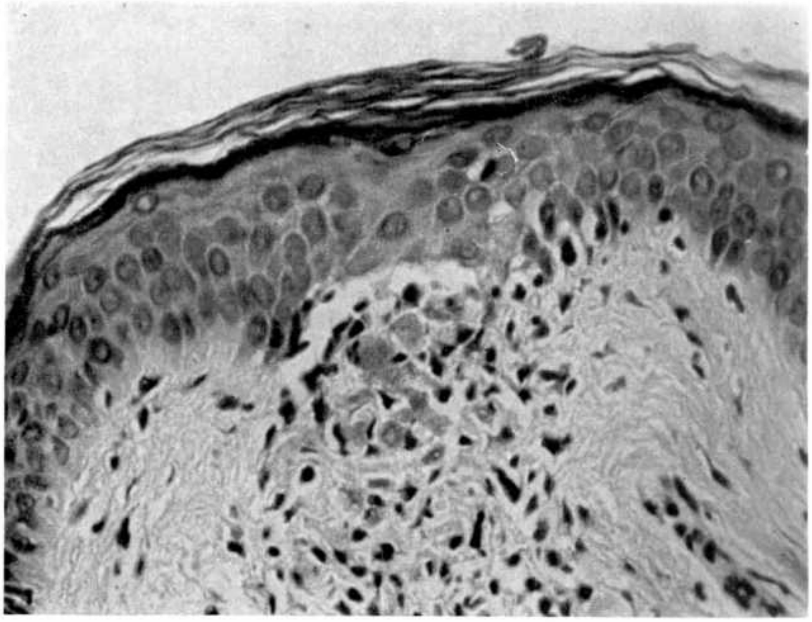

FIGURE 2. Anucleate phloxinophilic bodies situated above basement membrane, induced by scratching (Phloxin–tartrazine, × 375).

DISCUSSION

The cause of lichen planus is unknown (Black, 1972) despite attempts to ascertain its nature by examination of spontaneously formed eosinophilic bodies. The claims that these bodies are viral in origin (Thyresson & Moberger, 1957) have not been confirmed (Johnson & Fry, 1967; Sarkany & Gaylarde, 1970; Goltz & Hult, 1963). In contrast to eosinophilic bodies which form spontaneously, those induced by scratching can be examined at a known and determined age. The phloxinophilic nature of the latter may be characteristic of an earlier stage of development.

The delay in formation of eosinophilic bodies after scratching, their suppression by corticosteroids and the pronounced lymphocytic response favour a delayed hypersensitivity reaction, perhaps to an intracellular epidermal virus, the products of which are phloxinophilic. In a review of the aetiology of lichen planus, Midana & Ormea (1958) stated that some authors attach special importance to trauma, which is thought to act as a trigger factor actuating a latent infection. The present findings would support this view.

The induction of epidermal eosinophilic bodies by the trauma of scratching may prove to be a useful test in patients with active lichen planus-like eruptions, especially those induced by drugs.

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Wound healing in the oral mucosa of the acutely insulin deprived rat

MARK JOLLY AND NIGEL T.COOKE

Department of Oral Medicine and Oral Surgery, The University of Sydney,
Dental School, Sydney 2010, Australia

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SUMMARY

Standard wounds were produced on the palates of streptozotocin-induced 'diabetic' and normal rats. Healing, as estimated by the extent of epithelial migration after 24 h, was found to be identical in the two groups. The accumulation of glycogen and PAS positive, diastase-resistant material in the epithelium at the wound margins was far greater in the diabetic animals than in the controls. Indirect evidence is presented suggesting that the passage of glucose into epithelial cells is independent of insulin control.

The investigation was planned to assess two aspects of wound healing in the mouths of 'diabetic' rats compared with normal controls:

(1) The ability of epithelium to migrate across a wound.
(2) The accumulation of glycogen in the epithelium at the wound margins.

There is considerable disagreement about the effect of diabetes mellitus on wound healing. Williams (1968) defined the diabetic process as a relative or absolute deficiency of insulin. Thus, the diabetic state is purely a biochemical one. He stated that diabetes is responsible for a decrease in the rate of healing of wounds and attributed this to micro-angiopathic changes in the skin. Oakley, Pyke & Taylor (1968) held much the same view, whilst MacFarlane & Thomas (1966) mentioned only uncontrolled diabetes as a cause of defective wound healing. On the other hand, Wheelock & Marble (1971) considered that in most diabetic patients incisions will heal at a normal rate. In evaluating these papers it must be remembered that the injection of insulin does not mimic its physiological secretion by the pancreas, and that neither in experimental animals nor in man is it ever possible to attain complete control of the diabetic state. There is always some degree of physiological disturbance in the 'controlled' diabetic animal, including man.

Reports of glycogen in epithelium of skin and mucous membranes similarly have been confusing. The present authors believe that epithelium has the ability to synthesize glycogen. However, the glycogen concentration must reach a critical level before it is histochemically demonstrable. Using PAS staining techniques, Ohkawara & Halprin (1967) found that below levels of approximately 400 ng/mg of fresh tissue, glycogen could not be demonstrated; in the 400–500 ng/mg range it was sometimes seen and above 500 ng/mg it was always seen. The dynamic nature of glycogen and the fact that it is a storage substance also suggest that its existence in epithelium may be transitory and that its concentration, even in normal epithelium, may be variable.

Falin (1961) stated that the epithelium of the human hard palate contains very little, if any, glycogen, as shown by PAS staining, and that it is completely lacking in the oral epithelium of the rat. The latter observation suggested that the rat would be an excellent animal for the present investigation.

There have been many reports of glycogen appearing in epithelium following injury (Bradfield, 1951; Daniels, Brophy & Lobitz, 1961; Butcher & Klingsberg, 1962). Jolly (1964) found no evidence of the presence of glycogen in rat normal palatal epithelium, but considerable amounts were seen in the spinous layer adjacent to wound margins 24 h after wounding. In vitamin A deficient rats glycogen was almost completely absent from the wound margins, and healing was much delayed.

It would be of considerable interest and significance to know whether the storage of glucose as glycogen in epithelium is altered in the insulin deficient state and whether the normal accumulation of glycogen at healing wound margins is changed.

The second antemolar palatal ruga of the white rat (Kutuzov & Sicher, 1952) was chosen as the experimental site. This region has been used extensively by Gibbins (1962, 1968) as the site for producing a standardized oral mucosal wound. His technique and a 24 h healing period have proved eminently suitable for the study of migration of stratified squamous epithelium and have allowed a high level of reproducibility.

MATERIALS AND METHODS

Eight adult rats of the White Wistar strain, weighing between 200 and 300 g, were used. 4 days before the wounding experiment, four of the rats (two males and two females) were injected with freshly dissolved streptozotocin (65 mg/kg of body weight) into a tail vein to induce hyperglycaemia and 'diabetes mellitus'. The other four rats were used as controls. Following the injection of streptozotocin, all animals were maintained on the same diet and allowed free access to water.

The standard wound

The animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Sagatal; May and Baker). Using a scalpel handle specially constructed to hold two No. 11 Bard–Parker blades, parallel incisions 0·5–1·0 mm apart were made simultaneously in a coronal direction, along the anterior face of the second palatal antemolar ruga from one end of the ruga to the other. The incisions were made through the entire thickness of the epithelium and just into the corium. The resulting sliver of tissue was detached from its base using fine watchmakers' tweezers and the tip of another No. 11 Bard–Parker blade. Blood was collected from a tail vein for glucose estimation. The animals were later allowed normal food and water *ad libitum.*

After precisely 24 h the rats were anaesthetized and the palatal mucosa was removed as a single specimen and placed in Rossman's fluid (Emmel & Cowdry, 1964) as a fixative for glycogen. Rossman's fluid is claimed to give excellent results and has done so in the hands of the senior author. The fluid is stored in the refrigerator until use so that the specimen is placed in 'cold fixative', but it is not returned to the refrigerator. After fixation the palates were embedded to give sagittal sections.

Staining procedures

(1) Haematoxylin and eosin—to show epithelial migration.

(2) Periodic acid-Schiff reaction (PAS) with diastase control—to demonstrate glycogen and other polysaccharides.

TABLE 1. Blood glucose levels

Control		'Diabetic'	
Rat no.	Blood glucose (mg/100 ml)	Rat no.	Blood glucose (mg/100 ml)
1	108	5	296
2	114	6	397
3	106	7	206
4	138	8	341

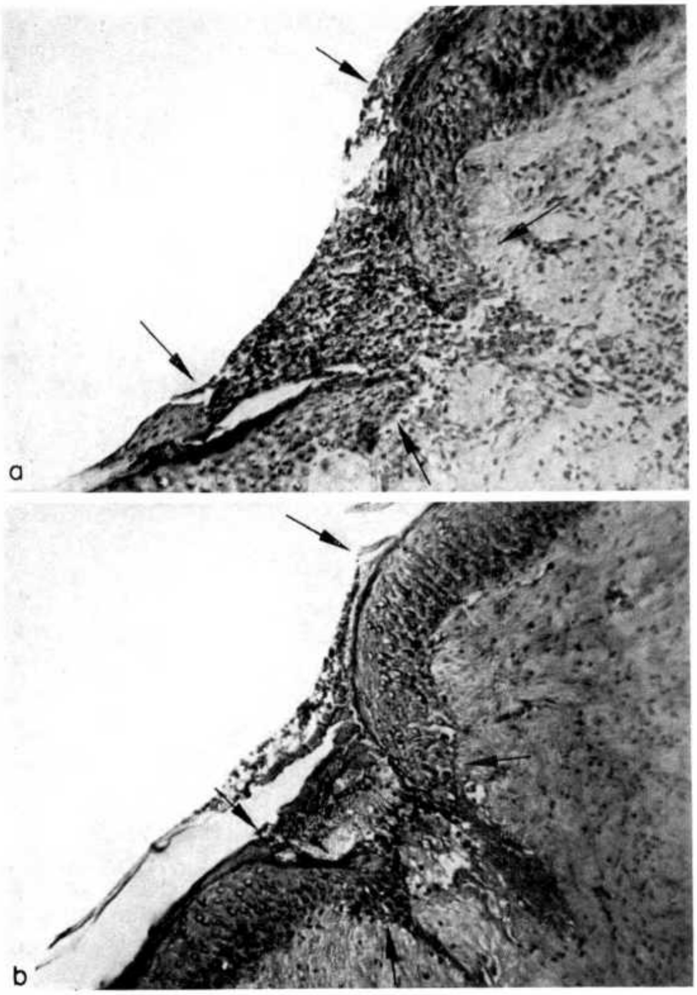

FIGURE 1. Wound healing at 24 h in a normal rat (a) and a 'diabetic' rat (b). The upper arrows point to the original wound margins and the lower arrows indicate the advancing epithelial buds. Epithelial migration was not retarded in the diabetic rats (H & E, × 80).

(3) PAS with dimedone blockade and diastase control—to facilitate the histochemical demonstration of glycogen.

The results of staining were assessed independently by the two authors using a six point scale.* When the assessments were compared there was a high level of agreement between the two; at no time did the ratings differ by more than one point.

RESULTS

The blood glucose of the control rats was within the normal range whilst that of the diabetic animals was significantly raised (Table 1).

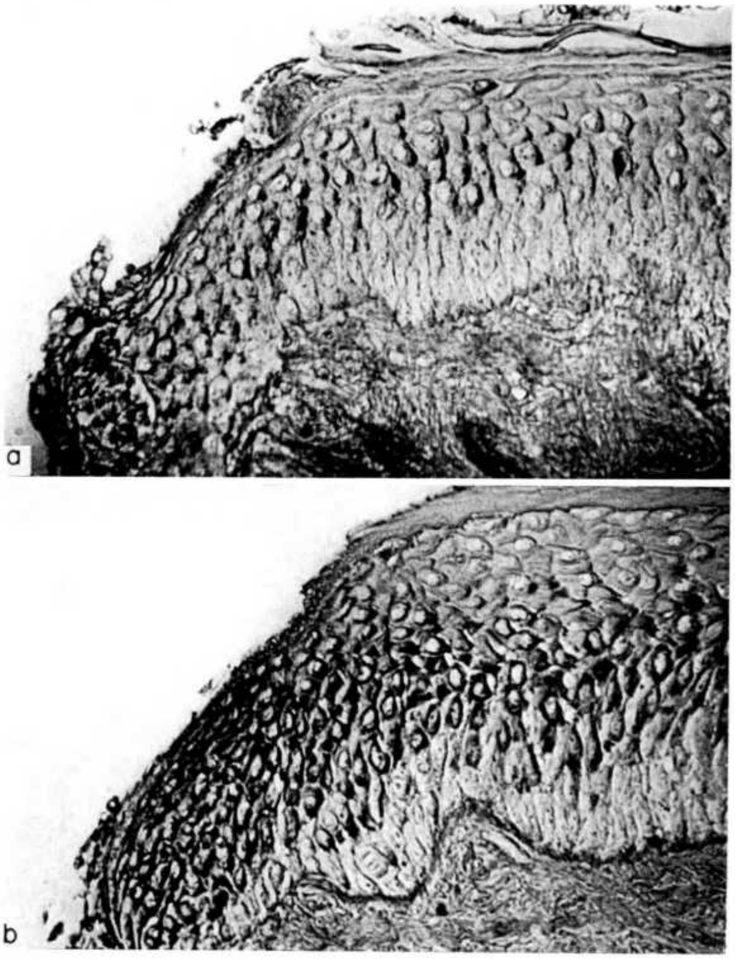

FIGURE 2. Corresponding wound margins from two rats at 24 h. PAS stain shows the presence of polysaccharide in the migrating epithelium, small deposits in the normal animal (a) and heavier and more extensive deposits in the 'diabetic' rat (b) (× 320).

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* −, complete lack of stain; ±, faint staining of a few cells and not constant in serial sections; +, light but definite staining of all cells in a particular region; + +, + + +, + + + +, indicating successively heavier and more extensive staining.

As all wounds were allowed to heal for precisely 24 h, the description of wound healing applies only to this stage of the repair process.

Wound healing

In both control and diabetic rats the bases of the wounds were covered by a typical clot, a fibrinous syncytium containing many leukocytes and macrophages. The epithelium that had started to bridge the wound appeared to have migrated from the adjacent basal and deep spinous layers. From each margin the migrating cells formed a 'bud' that progressed across the wound close to the connective tissue base and in contact with the clot.

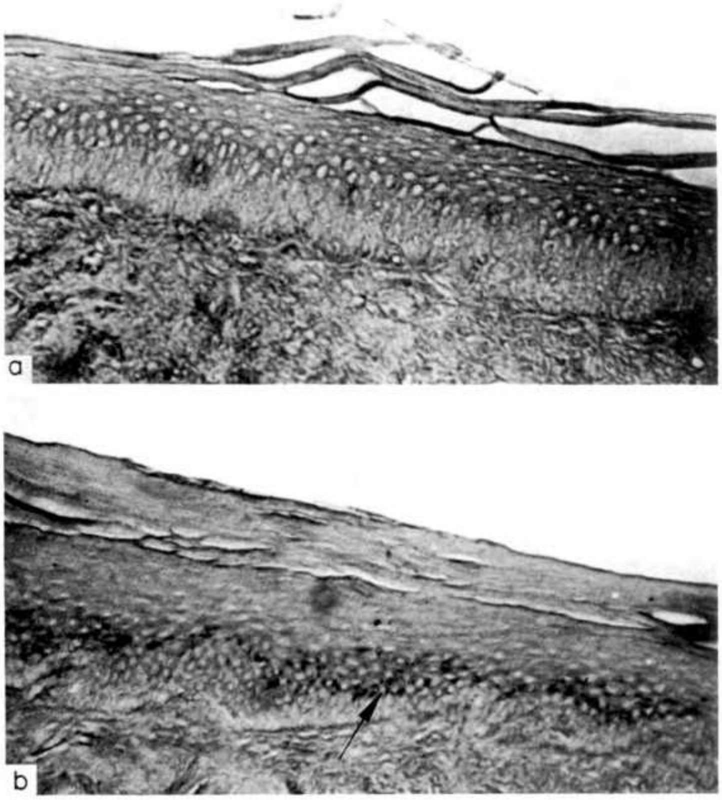

FIGURE 3. Palatal mucosa remote from the wound. In the normal rat (a) there is little or no evidence of polysaccharide in the epithelium, whilst in the 'diabetic' animal (b) there is an almost continuous layer (arrowed) as shown by PAS stain (× 80).

All wounds showed considerable healing in 24 h. The most interesting observation was that the extent of migration of epithelial cells in the diabetic rats was no less than in the normal controls (Fig. 1).

All the characteristics of the normal healing process were seen clearly, and in the sections stained with haematoxylin and eosin it was not possible to distinguish between those from the diabetic rats and those from the controls.

Glycogen identification

Almost all palatal epithelium away from the wounds in the control animals appeared to be devoid of glycogen, but in a few small, sporadic areas there was faint staining indicating the presence of glycogen. This was always confined to the mid-zone of the spinous layer.

Dimedone–PAS. In the control animals there were small accumulations of glycogen in the epithelium at the wound margins. It was mainly confined to the migrating epithelial cells, but in a few regions it was seen in the spinous layer for distances up to 1 mm from the original wound margins. At the wound margin this deposit generally occupied most of the width of the spinous layer, but further from the wound it tapered off to the middle and superficial layers, then to the middle layer only and finally it disappeared. In the 'diabetic' animals the pattern of distribution of glycogen was similar to that of the controls but it was heavier and extended further beyond the wound margins. Also, in the 'diabetic' animals it was not uncommon to find glycogen throughout much of the length of the spinous layer along the entire hard palate. This deposit was confined to the deep and middle layers of the stratum spinosum. There was no positive staining in the epithelium of either the 'diabetic' or the control animals following exposure of the sections to diastase.

PAS stain. Using PAS without dimedone, the extent and intensity of staining (presumably indicating glycogen and other polysaccharides) was greater than that seen after dimedone blockade. This applied to both 'diabetic' and normal rats, but staining was always darker and more extensive in the 'diabetic' animals than in the controls. In the 'diabetic' rats the accumulation of PAS positive material in the deep and middle layers of the stratum spinosum was almost continuous along the entire length of the hard palate (Figs. 2 and 3).

PAS after diastase. Much of the material stained by PAS alone was found to be diastase resistant, and presumably was not glycogen. The extent and intensity of staining after diastase digestion was as great or almost as great as that seen in sections not treated with diastase. Presumably the amount of PAS positive, diastase-resistant material was large compared with the amount of glycogen, or its affinity for PAS stain masked the removal of glycogen. The effectiveness of the diastase was checked using liver tissue from the same rats as a control, and glycogen was definitely removed.

DISCUSSION

Streptozotocin causes pancreatic islet beta-cell necrosis in rats, and the animals develop abnormally high blood sugar levels. Thus, insulin deficiency was induced in the rats used in this experiment, but it is important to note that it was of less than 4 days duration. Therefore, we were looking at wound healing in rats that had acute hyperglycaemia and impaired capacity to produce insulin, but in the absence of long term complications of diabetes mellitus.

Since there was no difference in the rate of migration of epithelium in wounds of 'diabetic' and normal rats, and at 24 h the two groups of wounds were indistinguishable, it seems that neither the lack of insulin nor the raised blood sugar levels of short duration affected the early healing process. Therefore, it is suggested that delayed healing in diabetes is due to a longer term effect of the diabetic state.

The histochemical findings in this investigation are in agreement with those of Kasahara (1964) who studied oral epithelium from human diabetics, and found that PAS positive material increased in buccal mucosal cells when carbohydrate metabolism was impaired and decreased with its correction. However, Kasahara suggested that most of the PAS positive material was glycogen, whereas our findings suggest that much of the PAS positive material in the 'diabetic' rats' mucosa was polysaccharide other than glycogen.

In the 'diabetic' animals there was evidence of increased glycogen and other PAS positive material at the wound margins and along the whole length of the epithelium of the hard palate. Although there are other possibilities, it is most likely that both the glycogen and the other polysaccharides are derived from glucose. If this is so, our results would seem to imply that, unlike muscle and adipose tissue, epithelium is not dependent upon an insulin controlled active transport system for the uptake of glucose. As in liver tissue, the concentration of circulating glucose would be the main factor determining its absorption. These findings agree with those of Schragger (1962), who produced evidence that the epidermal cell membrane poses no barrier to the passage of glucose and does not limit its utilization.

CONCLUSIONS

(1) In rats that had been deprived of insulin for less than 4 days the rate of healing of standard palatal wounds was no different from that in normal controls. Thus, neither lack of insulin nor raised blood sugar retarded epithelial migration.

(2) There was a greater accumulation of glycogen (as shown by Dimedone—PAS staining) in the epithelium at the wound margins in the 'diabetic' animals than in the controls.

(3) The accumulation of PAS positive, diastase-resistant material (presumably polysaccharide other than glycogen) in the epithelium at the wound margins was greater than the accumulation of glycogen in both the normal and the 'diabetic' animals. This was of greater amount and extent in the 'diabetic' animals than in the controls.

(4) Small amounts of glycogen and larger amounts of PAS positive, diastase-resistant material were found throughout much of the length of the spinous layer along the entire hard palate of the 'diabetic' rats, whilst insignificant amounts were seen in the controls.

(5) If glucose is the principal precursor of glycogen and other polysaccharides in epithelium, these results could be seen to show that the passage of glucose into the epithelial cell is independent of insulin.

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Identification and partial purification of chalone from human epidermis

DHARAM P.CHOPRA, LEONARD A.CHERKAS AND B.ALLEN FLAXMAN

Temple University Health Sciences Center, Department of Dermatology, The Skin and Cancer Hospital of Philadelphia, 3322 North Broad Street, Philadelphia, Pennsylvania 19140, U.S.A.

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SUMMARY

Crude human skin extracts having tissue-specific mitotic inhibitory activity (chalone), were studied by ethanol precipitation and disc gel electrophoresis. Precipitates obtained at various ethanol concentrations were dialyzed, lyophilized and tested for their mitotic inhibitory activity. It was found that the greatest inhibitory activity was concentrated in the precipitate formed in 81% ethanol, the active principle being concentrated about 200 times. Proteins in crude extracts were separated by disc gel electrophoresis, and at least eight bands were identified. Groups of bands were extracted from the electrophoretic gels and tested for their mitotic inhibitory activity. Fraction II, containing two bands, had the highest activity. Disc gel electrophoretic analysis of the 81% ethane precipitate revealed only two major bands, one of which could also be found in fraction II of the crude extract. This protein band, which is present in both of the mitotic inhibitory samples, may represent the human epidermal chalone.

Many investigators have described tissue-specific mitotic inhibitors (chalones) in non-human species (Bullough & Laurence, 1968; Chopra & Simnett, 1969; Rytomaa & Kiviniemi, 1968). Recently, we have demonstrated that an epidermal chalone is present in a crude extract of human skin (Chopra, Yu & Flaxman, 1972). This extract, when added to tissue cultures of normal or psoriatic human epidermal cells, caused a significant decrease in the number of mitoses due to a block in the G_2 part of the cell cycle (Chopra *et al.*, 1972; Chopra & Flaxman, 1973). Inhibition of mitosis was tissue specific, since the extract had no effect on kidney cell mitosis. Moreover, extracts from human liver prepared in a similar manner showed no inhibition of mitosis in epidermal cells (Chopra *et al.*, 1972). It has been proposed that chalones are regulators of cell proliferation under normal conditions (Bullough & Laurence, 1965). Reduction of chalone, which might occur in a number of ways, could lead to hyperproliferation characteristic of many benign and malignant tumors and of compensatory growth and wound healing. Further studies of chalone may, therefore, be of importance in understanding the pathogenesis of hyperproliferative conditions. While some progress has been made in purifying and characterizing epidermal chalone from other animal species (Hondius-Boldingh & Lawrence, 1968), it is important to perform similar studies of human epidermal chalone, since this substance may have special significance in understanding hyperproliferative conditions, such as psoriasis, which seem to occur only in humans. In the present study we report our progress towards partially purifying the epidermal chalone from human skin.

MATERIALS AND METHODS

Buffer extracts were prepared from human autopsy skin (4–24 h after death) according to a method previously described in detail (Chopra *et al.*, 1972). Lyophilized extracts prepared from each skin sample were stored separately at −20°C. Before further purification, the mitotic inhibitory activity of each crude extract was tested on cultures of normal human epidermal cells.

Ethanol precipitation of chalone from lyophilized skin extracts

The complete extraction procedure was carried out at 4–6°C. Lyophilized skin extract was dissolved in double distilled water at concentrations of 25 mg/ml. After filtration, 100% ethanol was added to the clear solution to give a final concentration of 50% ethanol. This was followed by stirring for 4–6 h then centrifugation of the resultant precipitate. Additional ethanol was then added to the supernatant fraction to give a concentration of 68% ethanol, followed again by centrifugation of the precipitate. The supernatant was further treated in a similar manner at 81% and 90% ethanol concentrations. Each isolated precipitate was washed in its respective ethanol concentration, centrifuged, dissolved in 0·02 M phosphate buffer (pH 7·5), dialyzed, lyophilized and stored at −20°C. Protein concentrations of the lyophilized precipitate were estimated by the method of Lowry *et al.* (1951).

Polyacrylamide disc electrophoresis

Several skin extracts and ethanol precipitates were analyzed by polyacrylamide disc electrophoresis performed in a Model 6 Canalco Apparatus (Canal Industrial Corporation, Rockville, Maryland), according to the method of Davis (1964) and Williams & Reisfeld (1964). For different electrophoretic runs, 150–600 μg of protein were applied to each gel. Electrophoresis in most cases was carried out at pH 9·2 and at a current of 1·5 mA for 2 h at room temperature. Under these conditions the tracking dye moved through approximately three-quarters of the gel. After completion of electrophoresis, gels were removed from the gel tubes by forcing water from a syringe between the gel and the glass walls of the tube. The gels were stained in amido black for 1 h and destained electrophoretically twice (20 min each time in 7·5% glacial acetic acid).

Cutting of electrophoretic gels

The procedure of cutting electrophoretic gels into individual bands has been used to obtain small amounts of relatively pure proteins (Maurer, 1971). However, the amount of protein in each band may be so small that to obtain any significant quantity, electrophoresis must be performed on a large number of tubes. Each extraction in the present work was carried out by cutting at least twenty-four gels, utilizing approximately 500 μg of total protein for each gel. No tracking dye was added to the buffer bath. To prevent denaturation of proteins, electrophoresis was carried out at 4°C. After completion of electrophoresis, gels were removed from the tubes, and, to examine the protein band pattern, one gel was stained in amido black. The remaining gels were then aligned in parallel fashion beside the stained preparation and cut into three parts, as shown in Fig. 1. Each group of gel portions, consisting of twenty-four pieces, was homogenized for 10 min in 10 ml of 0·02 M phosphate buffer (pH 7·5) and centrifuged at 18,000 rev/min for 20 min. The supernatant was removed and the residue re-extracted with buffer. The combined supernatants from each group were dialyzed separately (at 4°C) and lyophilized.

Cultures of epidermal cells as the test system

Epidermal cell cultures were made from normal human skin according to a method described previously (Flaxman, Lutzner & Van Scott, 1967). Most cell kinetic studies were performed on 7-day-old epidermal outgrowths which appeared around the explants. The effect of various test factors on mitotic index was studied by using the colcemid metaphase arrest technique. Test factors (crude skin extract, ethanol precipitates or gel extracts) and colcemid were added to the cultures, which were fixed in buffered formalin 4 h later. Explants were discarded and the outgrowths stained in haematoxylin for cytological examination. The MI (MI is the number of arrested metaphases per 1000 cells accumulated during the 4 h period of colcemid treatment) in the outgrowth was determined by a method described elsewhere (Chopra, 1971).

RESULTS

Effect of different samples of lyophilized crude skin extract on MI

Three samples of skin extracts were tested. As shown in Table 1, all three samples inhibited epidermal mitotic activity by more than 50%.

TABLE 1. Effects of three different samples of crude human skin extract on MI of normal human epidermal cells *in vitro*

Extract no.	Extract concentration (mg/ml)	MI ± SD		% depression	*P* value
		Control	Experimental		
1	15	23±5	10±2	57	<0·001
2	16	27±2	9±2	66	<0·001
3	12	18±2	5±1	68	<0·001

The effect of various ethanol extracts on MI

The ethanol extraction was carried out with a starting solution of 25 mg lyophilized skin extract per ml of distilled water. A total of 1500 mg of the crude skin extract was used, representing 600 mg of protein. The lyophilized fractions were added to the cultures at different concentrations. The results are shown in Table 2. The 50% and 90% ethanol fractions showed no significant mitotic inhibitory effect while the 68% fraction gave a slight depression of 29, 32 and 36% at protein concentrations of 15, 75 and 225 μg/ml respectively. The greatest inhibitory effect, however, was obtained with the 81% ethanol fraction (total of 3 mg protein obtained from 1500 mg of crude extract) where 75 and 225 μg protein per ml of culture fluid inhibited mitosis by 43% and 71% respectively.

Effect of different groups of skin proteins separated by disc gel electrophoresis on MI

Polyacrylamide disc gel electrophoresis of three different samples of crude skin extracts produced similar patterns of protein bands (Fig. 1). At least eight bands were present. The gels were cut into three fractions (I, II, III) (see Fig. 1) and the re-extracted protein added to the epidermal cultures. The results are shown in Table 3. Although the MI in control cultures (14±3) was unusually low in this experiment, comparison with the experimental values shows that only fraction II had a significant inhibitory effect on cell division. This experiment was repeated and the initial results confirmed. Fig. 1 shows that there are only two bands present in the inhibitory fraction II, one of which was also present following electrophoresis of the 81% ethanol precipitate (Ch.B., Fig. 2). Thus, the protein band Ch.B, which appears to be present in both the fraction II and 81% ethanol precipitate, may represent the human epidermal chalone.

TABLE 2. Effects of various ethanol precipitates on MI of normal human epidermal cells *in vitro*

μg protein per ml	Control MI ± SD	Precipitates obtained with							
		50% ethanol		68% ethanol		81% ethanol		90% ethanol	
		MI ± SD	depression	MI ± SD	depression	MI ± SD	depression	MI ± SD	depression
15	28 ± 5	24 ± 2	14	20 ± 2	29	26 ± 7	7	25 ± 6	11
75	28 ± 5	26 ± 2	7	19 ± 6	32 $P<0{\cdot}05$	16 ± 3	43 $P<0{\cdot}01$	27 ± 4	4
225	28 ± 5	24 ± 4	14	18 ± 3	36 $P<0{\cdot}025$	8 ± 3	71 $P<0{\cdot}001$	22 ± 5	21

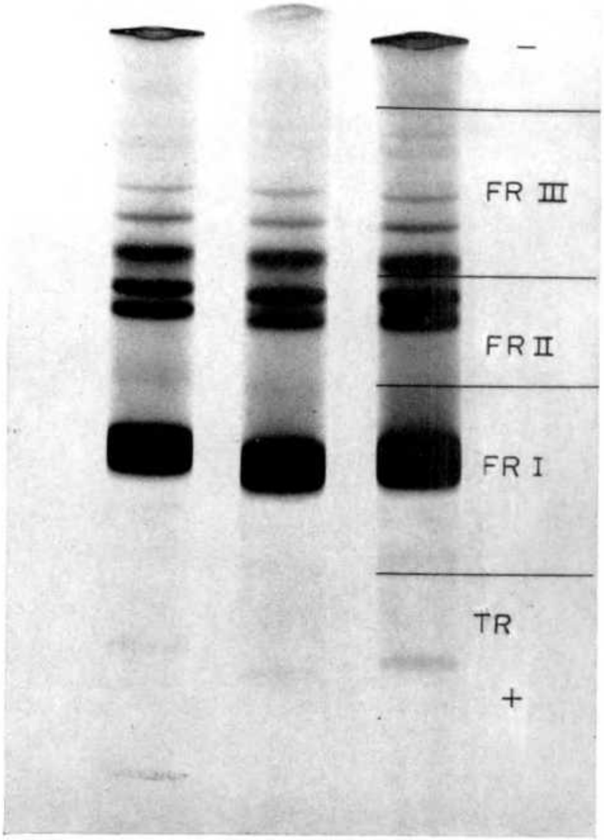

FIGURE 1. Polyacrylamide disc gel electrophoresis pattern of three different samples of mitotic inhibitory human skin extracts. The direction of migration was from the top to the bottom of the figure. FR I, II and III represent three groups of proteins extracted from electrophoretic gels. TR, tracking dye.

TABLE 3. Effect of different electrophoretic protein fractions (see Fig. 1) on MI of normal human epidermal cells *in vitro*

Fraction no.	μg protein/ml added to cultures	MI ± SD		% depression	*P* value
		Control	Treated		
I	100	14 ± 3	13 ± 1	7	< 0·1
II	100	14 ± 3	8 ± 2	43	< 0·05
III	100	14 ± 3	11 ± 3	21	< 0·1

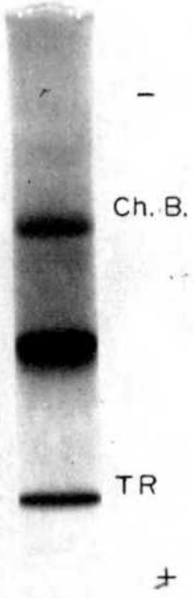

FIGURE 2. Polyacrylamide disc gel electrophoresis of 81% ethanol precipitate. The direction of migration was from the top to the bottom of the figure. TR, tracking dye; Ch.B., chalone band.

DISCUSSION

The present investigation demonstrates that the active epidermal mitotic inhibitor can be partially purified by ethanol precipitation and that its activity can be recovered from specific gel regions following electrophoresis. For ethanol precipitation of pig skin extract, Hondius-Boldingh & Laurence (1968) used a starting solution of 10 mg of skin extract per ml of distilled water, and reported that most of the epidermal chalone is concentrated in 70–80% ethanol. A more concentrated solution gave poor results. In our studies, although 81% ethanol concentrated most of the mitotic inhibitory activity, a starting solution of 25 mg/ml of human skin extract also gave satisfactory results. Some mitotic depression was also observed with the 68% ethanol precipitate (Table 2). This slight inhibitory effect of the 68% fraction could be due to a small amount of chalone that may have precipitated in it.

Further evidence that epidermal chalone activity was concentrated in the 81% precipitate was obtained from experiments where different groups of skin proteins were extracted from gels following electrophoresis of crude skin extract. The greatest inhibitory activity of epidermal mitosis was found in fraction II, which contained two protein bands (Fig. 1). Comparison of these bands with the electrophoretic bands obtained from the 81% ethanol fraction (Fig. 2) showed that both samples (fraction II and 81% ethanol precipitate) contained at least one protein in common. This protein may be the epidermal chalone. The degree to which the epidermal chalone was purified by ethanol extraction is difficult to determine precisely. From a starting material of 1500 mg skin extract (at least 600 mg protein) only 3 mg of protein was present in the 81% ethanol precipitate, the most active fraction.

Therefore it would seem that the chalone from the crude extract was concentrated about 200 times.

In some systems chalones act synergistically with adrenalin and hydrocortisone. For epidermal chalone, it has been shown that the inhibitor depresses mitosis in the mouse ear epidermis for only the first 5 h *in vitro* (Bullough & Laurence, 1968). The inhibitory effect could be restored if fresh adrenalin was added to the culture fluid. Similar results were reported by Hondius-Boldingh & Laurence (1968) for a partially purified epidermal chalone. It has been shown, however, that crude human skin extract inhibits mitosis of human epidermal cells *in vitro* in the absence of additional co-factors (Chopra *et al.*, 1972). In the present study similar results were obtained for the partially purified chalone from human skin extract. Regarding the thermolability of chalones, Hondius-Boldingh & Laurence (1968) reported that epidermal chalone remained active in aqueous solution for many days at 0°C and for several weeks at —20°C. For kidney chalone, it was found (Chopra & Simnett, 1969) that a lyophilized extract remained active for at least 16 weeks at —70°C. In the present investigation almost all samples of crude human skin extract or partially purified preparations were dialyzed and lyophilized, and remained active until they were exhausted over a period of 5–6 months. Therefore, it seems that lyophilization represents an excellent method of preserving chalone activity.

Because of the limited amounts of human skin available for experimental purposes, it was not possible to perform further biochemical studies of the active fractions. All the material obtained by the extraction procedure was tested for mitotic inhibitory activity. Some information regarding the chemical nature of pig epidermal chalone has been provided by Hondius-Boldingh & Laurence (1968), who believed that it is a glycoprotein with a molecular weight of 30–40,000. In spite of non-species-specificity of chalone, however, separate biochemical properties of human skin chalone must be investigated. Such studies might be of importance in understanding psoriasis, where the epidermal cells have been shown to respond to chalone from normal human skin (Chopra & Flaxman, 1973). The effectiveness of chalone from psoriatic skin has yet to be investigated.

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Porokeratosis of Mibelli after renal transplantation

A.L.MACMILLAN AND S.O.B.ROBERTS

Department of Dermatology, Addenbrooke's Hospital, Cambridge

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SUMMARY

A 50-year-old woman, who probably had pre-existing quiescent porokeratosis of Mibelli, suffered an abrupt and extensive eruption of porokeratosis in a systematized pattern 2 weeks after renal transplantation, while being treated with prednisolone and azathioprine.

Porokeratosis of Mibelli is a distinctive condition of the skin; although it was first described nearly 90 years ago, its aetiology and pathogenesis are still obscure. The case we report extends our knowledge of this disease by showing how it can behave following renal transplantation.

CASE REPORT

This patient, a married woman aged 51, clearly remembers having a few warty skin lesions in the left popliteal fossa and on the left calf, in childhood. At that time, cutaneous tuberculosis was diagnosed and freezing and surgical excision were employed with apparent success. One or two small papules may have remained but no new ones occurred during the next 40 years. In her late 40's, she developed progressive renal insufficiency and hypertension secondary to chronic pyelonephritis. Haemodialysis was started in December 1970, and on 31 January 1971 renal transplantation from a cadaver donor was performed. Detailed post-mortem examination of the donor showed no evidence of any skin disease.

Two weeks after the operation, and while on azathioprine 150 mg/day and prednisolone 75 mg/day, she began to develop numerous horny papules on the left lower limb from the buttock to the ankle. There were also a few lesions on the lower abdomen to the left of the midline. Some of the papules enlarged to a diameter of 1 cm and developed a massive horny epidermis; others became atrophic in the centre with a typical cornoid lamella peripherally (Fig. 1). In places their distribution was linear (Fig. 2). For about a month, new lesions continued to appear in profusion, and additional papules have developed up to the time of writing (October 1972). The main eruption is still confined to the left hind-quarter but a few lesions are present on the medial aspect of the right calf. Her present dose of azathioprine is 150 mg/day and of prednisolone 15 mg/day.

In the first 2 weeks after transplantation she complained of ulceration of the mouth confined to the right side of the hard palate. No infective cause was found, and healing occurred spontaneously. She also had during this period persistent herpes simplex of the right side of the lower lip. This eventually settled, but a crop of plane virus warts, which appeared on the back of the right hand a few weeks after transplantation, has persisted.

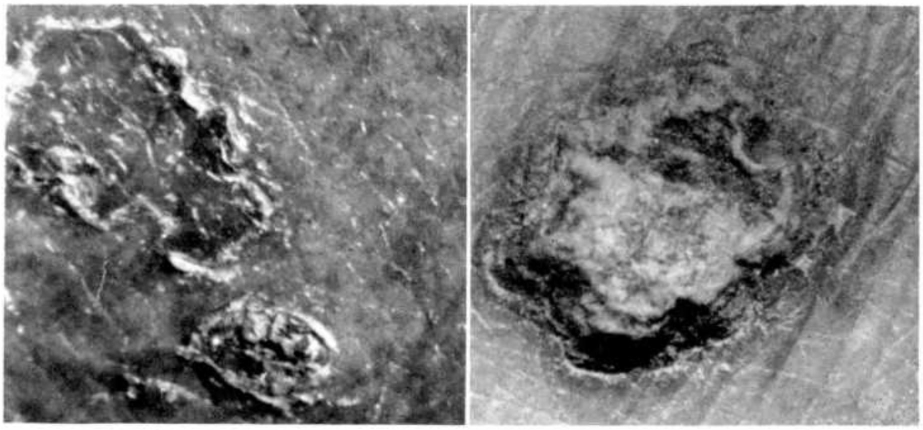

FIGURE 1. Hyperkeratotic and atrophic lesions of porokeratosis of Mibelli. Both lesions show the peripheral cornoid lamella as a fine single or multiple encircling ridge.

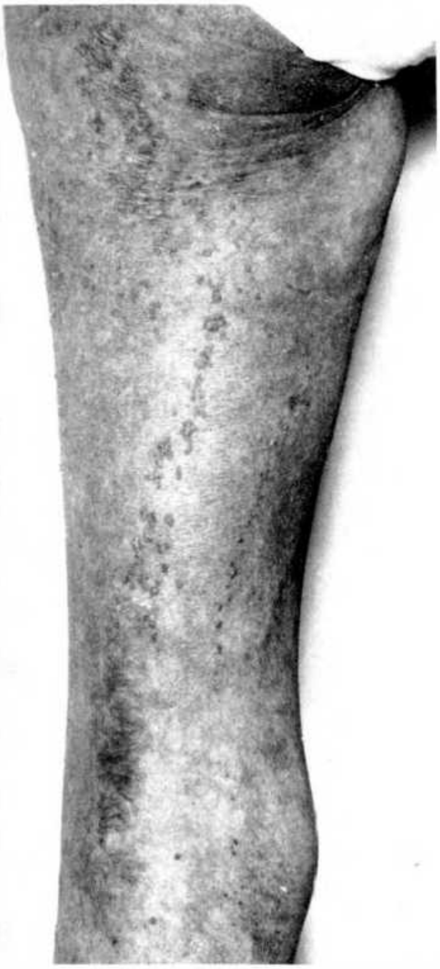

FIGURE 2. Linear lesions of porokeratosis of Mibelli on posterior thigh.

Family history

Although we have not been able to examine her relatives, enquiries by the patient suggest that none of her relations, including her two children, has any gross skin lesions.

Investigations

Biopsies of the lesions have shown the typical cornoid lamella of porokeratosis of Mibelli: a column of parakeratotic stratum corneum overlying a discrete depression of the epidermis, which at this site showed benign dyskeratosis and an underlying round cell infiltrate (Fig. 3). Towards the centre of the horny lesions there was hyperkeratosis with loss of epidermal appendages, and several unattached bundles of arrectores pili muscles were seen. No sections showed changes suggesting cutaneous tuberculosis.

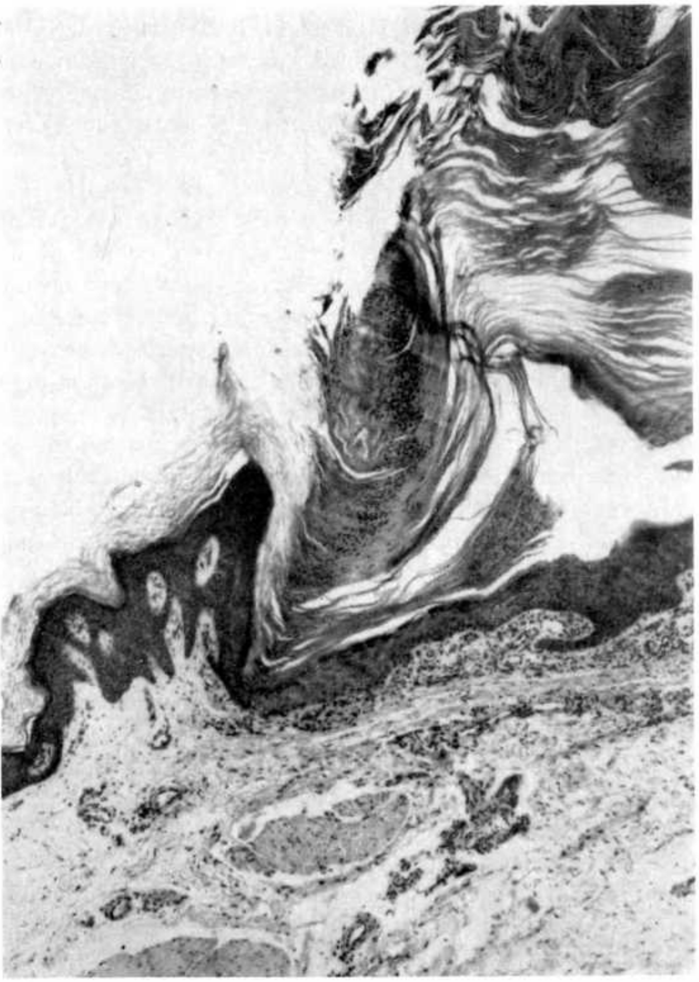

FIGURE 3. The cornoid lamella of parakeratotic keratin demarcates the normal epidermis on the left from the intralesional hyperkeratotic atrophic epidermis overlying dermis showing loss of appendages. At the site of the lamella the epidermis shows an indentation, vacuolated cells in the more superficial layers and benign dyskeratosis; below this there is a dermal lymphocytic infiltrate (H & E, × 40).

DISCUSSION

From the clinical and histological features there seems no doubt that the present eruption in this patient is porokeratosis of Mibelli occurring in a systematized pattern and showing some markedly hyperkeratotic lesions. The skin changes of childhood were, we suspect, also those of porokeratosis of Mibelli. Describing the features of 196 patients, Cockayne (1933) wrote, 'In all, the evolution of the disease has been extremely slow'. From the more recent literature it is equally clear that a sudden onset of profuse lesions of porokeratosis, or massive rapid extension of established porokeratosis previously of limited extent, is, even in early life, an extremely unusual event (Goldner, 1971; Jones & Smith, 1947; Mikhail & Wertheimer, 1968; Reed & Leone, 1970; Saunders, 1961). At the age of 51, it is most remarkable. We believe, therefore, that the eruption in our patient was not a fortuitous occurrence, but was probably provoked by events occurring about the time of renal transplantation.

The surgical procedure

Because this patient's kidney graft was placed in the left iliac fossa we have considered the possibility that surgical interference with the blood supply to the left lower limb might have influenced this eruption. Clinically there has been no sign since the operation of vascular disturbance affecting the left hind-quarter. Ischaemic episodes are not uncommon in the limbs of transplant recipients, related sometimes to arteriovenous shunts, sometimes to generalized arterial disease (Evans, 1971). In the Cambridge transplant unit, ischaemic complications of the legs have shown no preference for the side of the graft, apart from those cases where serious problems of vascular surgery arose and required the clamping of major vessels at operation (Evans, 1971). That was not the case in this patient, whose operation was performed uneventfully. The metabolic and immunodepressive aspects of the operation may have relevance here (Park *et al.*, 1971), but are likely to have been overshadowed by the immunosuppression of the pre-existing uraemic state (Wilson, Kirkpatrick and Talmage, 1965; Kroe & Vasquez, 1967) and of the post-operative drug therapy.

The reversal of renal failure

The metabolic transformation, which ultimately results from successful renal grafting, is usually not evident as early as 2 weeks after transplantation, nor was it in this patient. It seems unlikely, therefore, that this is relevant.

Mibelli originally described porokeratosis as a disease of sweat ducts, and Saunders as late as 1961 supported this view. There is anhidrosis within the lesions but the histochemical evidence upon which Saunders based his opinion has been convincingly refuted (Mikhail & Wertheimer, 1968). Because of this, and because of the occasional involvement of the mucous membranes by porokeratosis, the sweat duct is no longer regarded as having a central role in this process. In uraemia, many patients recognise that their skin is drier and that sweating is impaired. Successful renal transplantation is usually followed by an increased capacity to sweat, which the patient often notices.

Although the sweat glands are reportedly smaller than normal in uraemia (Crawley, Hoch-Ligetic & Bond, 1961), we know of no systematic studies of sweat gland structure or function after transplantation. Our impression is that improvement is a gradual process, not far advanced in 2 weeks. Therefore, it is improbable that changes in sweat glands and ducts were relevant in the aetiology of this patient's porokeratosis. In fact we see nothing in this case to direct attention back to the sweat duct in porokeratosis.

The donor kidney

It has been suggested that the large amount of foreign antigen which the grafted kidney represents may be a factor in inducing reticulum cell sarcoma, a tumour which is unexpectedly prevalent in renal transplant recipients (Doak *et al.*, 1968; Anonymous, 1972). Even if this is true, there seems little reason to assume that because a process is capable of inducing reticulo-endothelial neoplasia it is also likely to be a cause of a benign epithelial proliferation.

The donor kidney could conceivably be carrying an infective agent—perhaps a virus (Gardner *et al.*, 1971). There is scant evidence supporting an infective aetiology for porokeratosis (Savage & Lederer, 1951), but modern virological techniques have not been used, and this question must be regarded as open. The graft recipient is assumed to have had porokeratosis already, but a further inoculation of a causative virus might be a factor in invoking a widespread recrudescence of her quiescent disease. We have no evidence for or against this, except that the donor, a female aged 20, had no sign of porokeratosis according to the hospital notes and the autopsy report.

Immunosuppressive therapy

Azathioprine and prednisolone may influence the skin by direct action, or indirectly by impairing the body's immune resistance to micro-organisms capable of producing cutaneous pathology. Prednisolone, in the doses used here, almost certainly depressed the mitotic rate of the normal epidermis (Bullough & Laurence, 1968; Fisher & Maibach, 1971; Marks *et al.*, 1971). Azathioprine probably has a similar effect. The lesions of porokeratosis seem not to be influenced by corticosteroids, even in the high doses achieved with intradermal infiltration of triamcinolone suspension (Allenby, 1972). Reed & Leone (1970) have suggested that porokeratosis represents a clone of mutant (abnormal) epidermal cells capable of spreading outwards at the expense of the normal epidermis. If this concept is true, we presume that the abnormal epidermal cells have been present in minute numbers throughout life in all the areas now affected in this patient. In childhood, in one limited area, they multiplied to the detriment of the normal epidermal cells. In other areas (mainly the left leg) these mutant cells persisted but were held in check, perhaps by adjacent normal epidermal cells, perhaps by immune mechanisms as envisaged in the theory of immunosurveillance (Keast, 1970). Conceivably the phenomenon of contact inhibition of one cell by another, known as allogeneic inhibition (Hellstrom, 1966), might be important. The mechanisms by which such inhibition is achieved remain largely obscure, and were apparently not suppressed by steroids or azathioprine in one experiment where this was tested (Moller *et al.*, 1967). If the mitotic rate of normal epidermal cells were suppressed by the drugs used and if the mutant cells remained uninfluenced, it might, regardless of the mode of previous inhibition, leave the porokeratosis cells at an advantage and lead to the development of clinically evident lesions. The lymphocytic infiltrate normally found beneath the cornoid lamella suggests that an immune response is involved in the established lesions of porokeratosis. Whether classical immune mechanisms have a role in the pre-eruptive phase is not known. If they are implicated, this patient's eruption could be explained simply by the impairment of this immune response. The presence of the typical lymphocytic infiltrate in biopsies from our patient shows that this response, though perhaps impaired, was not absent.

As already stated, the possibility that an infective agent has some rôle in porokeratosis has not yet been completely excluded and is in no way incompatible with the mutant clone hypothesis. Even those cases with a positive family history and an apparent dominant inheritance could be explained if the agent were a virus capable of vertical transmission. In this case a virus, long quiescent, could have been released from immune control by immunosuppressive therapy just as, for example, the varicella-zoster virus often is (Evans, 1971). The patient gave a history of herpes simplex lesions and did develop after transplantation herpes simplex of the lip as well as a crop of virus warts. The rapid eruption of new lesions while the patient was receiving high doses of steroids, and the much slower progression on lower doses, is compatible with an infective cause.

One other aspect of considerable importance to the patient remains to be considered. Epidermal neoplasms, squamous cell carcinoma and basal cell carcinoma are almost certainly more common and probably occur at an earlier age in renal transplant recipients than in normal subjects. Most reported cases have, however, come from sunny regions, where solar damage to the skin is common (Penn, 1970; Walder, Robertson & Jeremy, 1971). In Cambridge, the only epidermal neoplasm so far observed has been a squamous carcinoma occurring in a South African patient. A continuing study by one of us (S.O.B.R.), of skin changes in over seventy survivors of transplantation, who attend the Cambridge transplant follow-up clinic, has so far revealed only two patients with solar keratoses, neither with neoplasia at present. We agree with the suggestion that transplant patients who have solar damage of the skin, especially with keratoses, are most at risk. We wonder whether other pre-malignant skin conditions present a similar hazard. Porokeratosis is occasionally complicated by the development of squamous carcinoma in the affected skin (Savage, 1964; Johnson, 1958; Ehlers & Rothe, 1971; Oberste-Lehn & Moll, 1968). We suspect that this patient has an even greater risk of developing this complication, and we therefore plan careful surveillance.

CONCLUSIONS

The patient we describe has had a recrudescence and rapid extension of porokeratosis of Mibelli many years after the appearance of the initial lesions. This extension occurred 2 weeks after renal transplantation and was most probably related to the immunosuppressive therapy. We accept the suggestion that in porokeratosis a clone of abnormal cells exists in the epidermis. Inhibitory forces, exerted by adjacent normal epidermal cells, or by classical immune mechanisms, may contain the cell mass of the mutant clone, and prevent macroscopic lesions from developing. Immunosuppressive therapy by impairing either or both of these processes may have led to the events we have described. Alternatively, immunosuppression may have allowed an infective agent to proliferate.

Whatever the mechanism, any comprehensive theory of the aetiology and pathology of porokeratosis of Mibelli should include an explanation of this observation. Because porokeratosis carries an increased risk of epidermal neoplasia and because immunosuppression in the presence of pre-malignant epidermal states induced by sun damage seems also to increase this risk, we believe our patient is particularly likely to develop squamous carcinoma of the skin.

ACKNOWLEDGMENTS

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In vitro regeneration of the barrier to water penetration in human epidermis

J.P.ASTLEY AND M.LEVINE

Department of Biological Chemistry, University of Manchester, Manchester M13 9PL

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SUMMARY

A method is presented for simultaneous *in vitro* growth and measurement of permeability of human skin. During culture of specimens, the parakeratotic layer formed is shown to possess considerable barrier function. Maximum barrier capacity of this layer *in vitro* is attained at 14–17 days' incubation. When the stratum corneum is removed prior to incubation, the maximum barrier capacity is reached in 3–4 days. The difference in behaviour between intact and stripped skin is reflected in the pattern of DNA synthesis of the basal cells, which show an enhanced proliferation after stripping. Barrier regeneration of stripped skin is seen closely to resemble the parallel situation *in vivo*.

A major function of epidermis, and possibly its most important, is the prevention of water loss and exclusion of foreign materials from the body. The stratum corneum provides the barrier to penetration, and measurement of its permeability is a reliable quantitative parameter for assessment of integrity. A number of workers have used penetration rates to study regeneration of the barrier *in vivo* after its removal by tape stripping. Malkinson (1958) measured the permeability of human skin to ^{14}C testosterone by loss of applied radioactivity from the skin surface; a method of limited accuracy. Monash & Blank (1958), Matoltsy, Schragger & Matoltsy (1962) and Spruit & Malten (1966) ascertained permeability by measurement of water loss from the skin surface. Although a more reliable method than that employed by Malkinson, the *in vivo* technique remains limited in the degree of experimental control attainable. Greater regulation is possible in an *in vitro* system, although its own particular limitations must be taken into account.

The cultivation of explants of human skin *in vitro* has been reported by a number of workers, including Hambrick, Lamberg & Bloomberg (1967), Levine (1972), Reaven & Cox (1965, 1968), Reinertson (1961) and Sarkany, Grice & Caron (1965). The criteria employed to assess the condition of the cultured skin include histological examination, incorporation of radioactively labelled metabolic precursors, and histochemical studies. Sarkany *et al.* (1965) included the formation of a horny layer, as judged by histological examination, in their criteria for growth.

In this report we present a method for determining the barrier function of the new horny layer as it is regenerated *in vitro*. Since the formation of the barrier layer is the final stage in the maturation of epidermal cells, information is also obtained about the condition of the epidermis as a whole. The permeability of the regenerating layer has been measured both in the presence of the original stratum corneum and following its removal prior to culture, regeneration being enhanced after stripping. The pattern of growth after removal of the stratum corneum resembles the parallel situation *in vivo* for the first few days. Associated with barrier renewal are corresponding changes in histological appearance and cell proliferation, as judged by autoradiographic studies.

In the model system described here, changes in barrier function brought about by stimuli or damage can be monitored quantitatively, and studied in relation to epidermal regeneration as a whole.

MATERIALS AND METHODS

Culture media

The basic synthetic medium used was Eagle's Minimal Essential Medium (MEM) (Wellcome Research, Beckenham, Kent), to which were routinely added streptomycin sulphate (40 μg/ml), penicillin G (100 units/ml), mycostatin (E. R. Squibb and Sons Ltd, London) (100 units/ml) and 10% human serum. Some specimens were maintained in phosphate buffered isotonic saline (pH 7·0) with antibiotics added as in the growth medium.

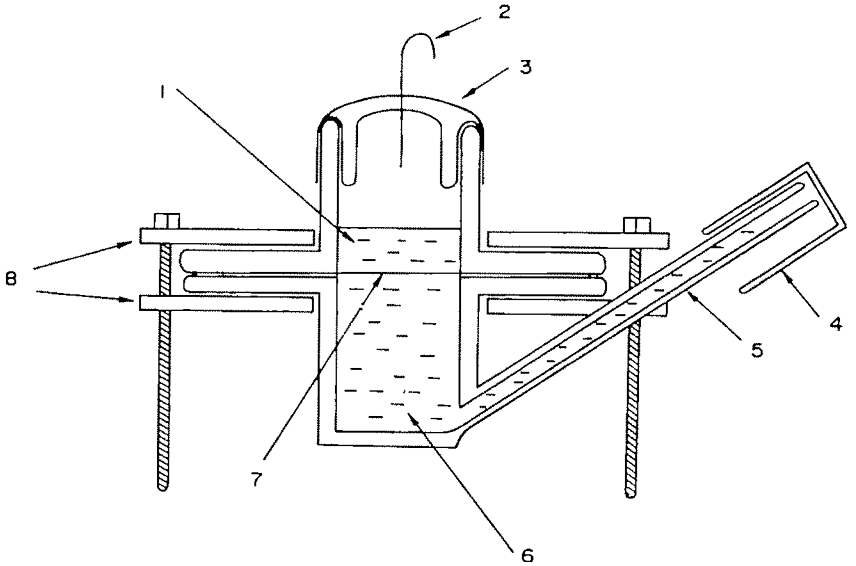

FIGURE 1. Diffusion cell. 1, epidermal chamber; 2, hypodermic needle; 3, serum cap; 4, small glass beaker; 5, side arm; 6, dermal chamber; 7, skin clamped between flanges; 8, brass clamps.

Organ culture

Specimens of adult human skin were obtained from amputated limbs and occasionally post mortem. Donors were of either sex, predominantly in the 50–80 years' age group. Specimens were taken from the thigh or lower leg with a dermatome (Davol Ltd, Providence, Rhode Island) set at 0·38 mm thickness. The site was first prepared by liberal swabbing with a solution of 0·5% chlorhexidine in 70% aqueous ethanol, followed by aqueous ethanol on a sterile swab. The excised sheets of skin were placed epidermal side down in petri dishes, on gauze moistened with Hank's balanced salt solution (containing 100 units/ml penicillin G and 40 μg/ml streptomycin). For some later work full-thickness skin was removed using a scalpel. The specimens were kept at 4°C until required for use (less than 6 days). No effect of such storage time upon subsequent behaviour of skin was observed.

During culture the skin was supported, epidermal side upward, in diffusion cells, as illustrated in Fig. 1. An efficient seal between compartments was obtained by applying a thin layer of vacuum grease (Silicone High Vacuum Grease, Edwards High Vacuum Ltd) around the edge of the skin before the flanges were clamped together, care being taken that no grease came into contact with the exposed surface of the skin. The lower (dermal) compartment was filled with growth medium, thus maintaining contact between the dermis of the skin specimen and the medium. The epidermal surface was exposed to the atmosphere in the incubator, which was maintained at constant temperature (usually 32°C) and 75% relative humidity. The medium was equilibrated in a 95% air, 5% CO_2 atmosphere giving a pH of $7{\cdot}2 \pm 0{\cdot}1$.

In addition to cultures maintained in the diffusion cells, explants were incubated using the method described by Levine (1972), and removed at intervals during the experimental period. These 'control' specimens served to provide a reference for comparison of permeability changes with growth.

Measurement of permeability

Permeability was measured during growth of the cultures. Tritiated water (5 mCi/ml, Radiochemical Centre, Amersham) was the penetrant used, and a solution containing 80 μCi/ml was prepared in the same type of medium as that bathing the dermis. A known volume (0·5 or 1·0 ml) was applied to the epidermal side, the solution levels were balanced by addition of extra medium to the dermal compartment, and then the cells were placed in the incubator. Samples (0·2 ml) were removed from the dermal compartment at measured time intervals and replaced with an equivalent volume of the relevant medium. The sample was transferred to a vial and scintillator added (0·4% PPO, 0·04% POPOP in xylene/ethanol 68/30). The concentrations of tritiated water above and below the skin were determined by liquid scintillation counting in a Packard Tri-Carb liquid scintillation spectrometer. A rigorous method of calculation, involving correction for the samples removed was employed (Appendix 1).

Treatment of tissue before incubation

Removal of the stratum corneum was achieved by successive stripping of the skin with adhesive tape (Ofrex Ltd, Manchester). The skin was placed on a piece of gauze over a stainless steel block and clamped into position by means of an adjustable frame (Rudolph & Linnevold, 1972). The tissue was held firmly in place during the stripping. The cells adhering to the tape were stained in eosin and viewed under the light microscope. With successive strippings the number of cells was observed to decrease and stripping was discontinued when few stained cells remained.

The method of stripping was modified for later experiments to avoid tearing the skin as the tape was removed. Full-thickness skin was obtained and clamped into position on the block. The stratum corneum was completely stripped from it, then a slice of split-thickness was removed with the dermatome and placed in position on the diffusion cell.

Histology and autoradiography

Specimens removed from the diffusion cells at the end of the experiment, and 'control' specimens harvested at intervals during the experiment, were fixed in 10% neutral formalin. After dehydration the tissues were embedded in paraplast wax, serial sections cut at 4 μm and stained in haematoxylin and eosin.

Some 'control' cultures and one set of specimens in diffusion cells were labelled with tritiated thymidine (thymidine 6-T) 2·5 Ci/mM (Radiochemical Centre, Amersham), at a concentration of 1 μCi/ml medium, for 24 h before termination of culture. Up to the staining stage the only departure from normal histological procedure was the use of subbed slides (prepared by coating slides with a mixture of gelatin and chrome alum). The stripping film technique was employed using Kodak AR 10 nuclear emulsion. The specimens were exposed for 1 week at 4°C then developed (6 min), rinsed (30 s) and fixed (12 min) at 20°C. Excess emulsion was trimmed away and the specimens stained in haemalum.

RESULTS AND DISCUSSION

Culture of unstripped skin

The results of two experiments, in which permeability was followed for 21 and 24 days *in vitro* growth, are given in Table 1 (a) and (b), and data of Table 1 (a) shown graphically in Fig. 2. By maintaining some specimens in buffered saline the permeability in the absence of growth was also followed.

TABLE 1. The effect of growth *in vitro* on the permeability of unstripped human skin in tritiated water

Time (days)	Growth medium throughout experiment $P \times 10^7$ (i)	Growth medium throughout experiment $P \times 10^7$ (ii)	Buffered saline throughout experiment $P_s(t) \times 10^7$	Growth medium but buffered saline on test days $P \times 10^7$ (iii)
(a)*				
1	3·18±0·13	4·20±0·21	3·76±0·12	4·41±0·31
3	2·97±0·16	4·16±0·10	3·65±0·08	3·75±0·13
6	3·50±0·43	4·02±0·25	4·12±0·26	4·59±0·27
10	3·33±0·12	4·41±0·17	5·67±0·17	4·94±0·46
14	3·01±0·17	4·50±0·13	6·36±0·27	4·42±0·76
17	3·55±0·16	4·41±0·22	6·43±0·15	4·98±0·37
21	3·46±0·10	4·97±0·32	7·79±0·21	5·65±0·50
(b)				
1	4·08±0·12	4·07±0·20	4·22±0·48	3·52±0·28
3	4·65±0·13	4·65±0·29	6·10±0·15	3·80±0·09
6	6·86±0·72	5·13±0·29	5·16±0·11	4·96±0·12
10	7·55±1·79	5·98±1·54	8·07±2·09	7·77±2·08
14	6·08±0·22	6·55±0·08	8·43±0·24	7·14±0·18
17	6·94±0·28	7·05±0·35	13·01±0·28	8·85±0·40
21	8·00±0·25	8·20±0·86	16·45±1·70	9·69±0·67
24	7·44±0·34	7·65±0·42	26·30±1·14	9·91±0·32

Permeability coefficients (P) given in cm/s. Errors quoted as ±95% confidence limits (n = 6 where n is the number of samples taken to calculate each permeability coefficient). (a) and (b) refer to duplicate experiments. Measurements carried out at 32°C.

*The data in (a) are represented graphically in Fig. 2.

All specimens showed a significant increase in permeability over the test period. Although a substantial increase was observed in one experiment (Table 1(b)) for skin incubated in growth medium, there was a correspondingly greater increase in specimens exposed to buffered saline and buffered saline/growth medium, the behaviour of the latter being intermediate. While the skin specimens for different experiments were obtained from the thigh region, they were not obtained from the same subject, and comparison of absolute values between different experiments is not valid. It thus appears that the explants in growth medium have maintained their barrier function to a far greater extent than those in saline.

Histological examination of skin removed from the diffusion cells and of 'control' specimens maintained in culture on nylon mesh supports in petri dishes revealed that specimens incubated in growth medium exhibited the generally observed pattern of *in vitro* growth. There was a gradual flattening and thinning of the Malpighian cell layer, the appearance of a parakeratotic layer beneath the original stratum corneum and, at a later stage, pockets of regenerated Malpighian cells beneath the keratinized layer. Specimens exposed alternately to growth medium and to buffered saline had a thinner parakeratotic layer, while those in buffered isotonic saline showed no growth. Correlation of permeability with the histological picture indicates that the parakeratotic layer is responsible for maintenance of the barrier. Its impermeability is not as effective as that of the original stratum corneum, since in spite of its presence a significant increase in permeability is observed. From the data it is possible to arrive at a quantitative estimate for the permeability of the parakeratotic tissue. The method of calculation is outlined in Appendix 2 and Table 2, and values obtained are given in Table 3.

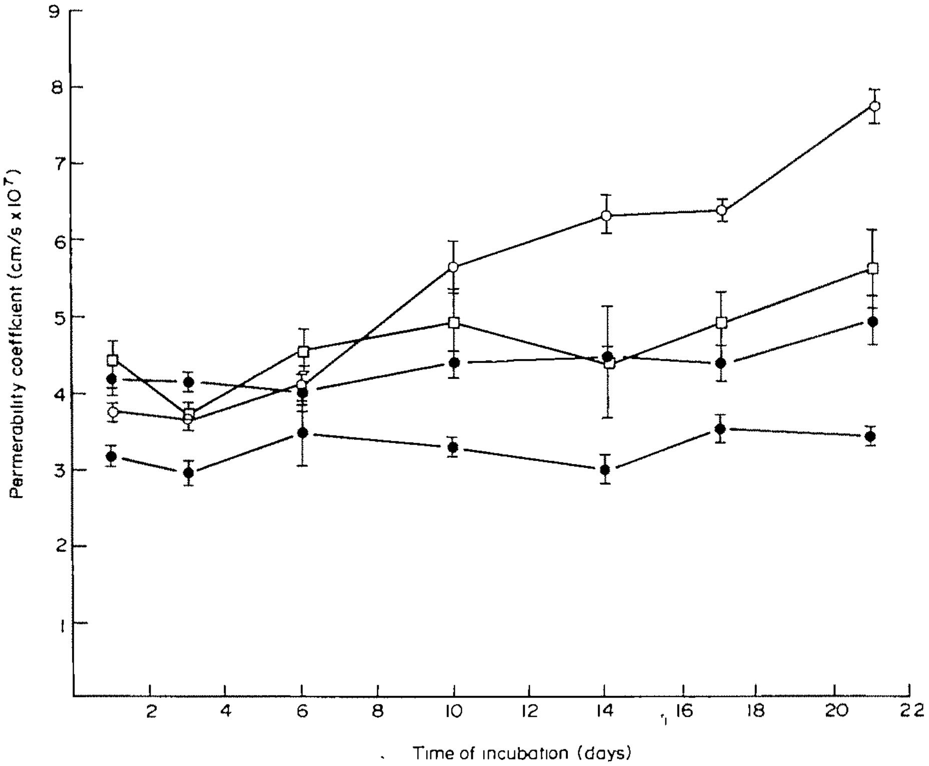

FIGURE 2. Effect of growth *in vitro* upon permeability of unstripped human skin. ●, specimens in growth medium; □, specimen in growth medium except on 'test' days when measurements were carried out in buffered saline; ○, specimen in buffered saline. Errors are plotted as ±95% confidence intervals.

We can conclude that the parakeratotic layer has considerable barrier function with permeability about four-fold greater than that of normal stratum corneum. This value is attained after 14–17 days incubation. Specimens exposed to both growth medium and buffered saline appear to have a less effective barrier, but this is probably due to the presence of a thinner parakeratotic layer.

TABLE 2. Permeability coefficients of parakeratotic tissue $P_2(t)$ (cm/s × 10⁷)

Permeability coefficient in saline (Ps) (cm/s × 10⁷)	Ratio $\frac{Ps(t)}{Ps(o)}$	Permeability coefficient of skin in medium (P) (cm/s × 10⁷)	1/P	$P_1(t) = (Ps(t)/Ps(o)) \times P_1(o)$	$1/P_1(t)$	$1/P_2(t)$	$P_2(t)$ (cm/s × 10⁷)
(i)							
(1)* 3·76	1·0	3·18	0·3145	3·180	0·3145	—	—
(3) 3·65	0·970	2·97	0·3367	3·085	0·3241	—	—
(6) 4·12	1·096	3·50	0·2857	3·485	0·2869	—	—
(10) 5·67	1·508	3·33	0·3003	4·795	0·2086	0·0917	10·9
(14) 6·36	1·691	3·01	0·3322	5·377	0·1860	0·1462	6·8
(17) 6·43	1·710	3·55	0·2817	5·438	0·1839	0·0978	10·2
(21) 7·79	2·072	3·46	0·2890	6·589	0·1518	0·1372	7·3

Calculated from the data of Table 1a(i). Method of calculation is outlined in Appendix II.
* Time (days) of measurements in brackets.

TABLE 3. Permeability coefficients of parakeratotic tissue ($P_2(t)$ (cm/s × 10⁷))

Time of culture (days)	Permeability coefficients of parakeratotic tissue					
	Data from Table 1a			Data from Table 1b		
	(i)	(ii)	(iii)	(i)	(ii)	(iii)
1	—	—	—	—	—	—
3	—	—	30·4	—	22·2	—
6	—	31·6	90·9	—	99·0	—
10	10·9	14·5	19·2	232·5	25·8	—
14	6·8	12·3	10·9	24·3	34·5	—
17	10·2	11·4	14·7	15·5	16·1	41·0
21	7·3	11·6	14·8	16·1	16·9	32·9
24	—	—	—	10·5	10·9	18·1

Calculated from the data of Table 1a, b (method in Appendix II and example in Table 2). (i) and (ii) are specimens cultured in growth medium; (iii) specimen cultured alternately in growth medium and saline.

Culture of stripped skin

The results of three experiments in which *in vitro* growth was maintained for 8, 11 and 25 days are given in Tables 4, 5 and 6. Table 5 data are expressed graphically in Fig. 3. The stripped specimens maintained in buffered saline served to monitor permeability behaviour in the absence of *in vitro* growth, while the unstripped specimens maintained in growth medium served as a reference for comparison.

The variation in initial permeability of the stripped skin (Tables 4 and 6) was due to variable removal of the stratum corneum as judged histologically. Maximum values appear to correlate with complete removal. Thus an approximately fifty-fold increase in permeability is observed upon stripping. However, in one experiment (Table 5), although the histology indicated complete removal, the values were lower than usual because of the diffusional resistance of a full-thickness dermis present in the explants. The permeability coefficients of the stripped specimens exposed to buffered saline remained relatively constant, although there appeared to be a slight decrease at 1 and 2 days, which may be due to some barrier regeneration. Histological examination, however, revealed no discernible growth.

TABLE 4. Regeneration of stripped skin, *in vitro*, as measured by its permeability to tritiated water

Time (days)	Stripped skin in buffered isotonic saline pH 7·0	Stripped skin 1st day in saline, then growth medium	Stripped skin in growth medium		Unstripped skin in growth medium
			(a)*	(b)*	
	(n = 6)	(n = 6)	(n = 6)	(n = 6)	(n = 6)
0	29·3±8·3	40·9±17·8	249·6±35†	148·5±26†	3·81±1·1
1	26·8±7·2	23·8±3·9	149·0±20	89·5±3·9	3·40±0·53
2	18·9±14·5	20·1±1·9	104·6±13	64·6±3·5	4·05±0·34
3	29·8±8·8	17·8±3·4	74·9±14	46·0±6	4·30±0·92
4	37·1±4·9	19·1±5·0	64·8±7·5	40·8±2·1	4·25±0·43
8	49·9±36·7	15·4±1·6	58·1±5·7	31·0±2·7	4·21±0·16

Permeability coefficients (P) are given in cm/s × 10^7. n = number of samples taken to calculate each permeability coefficient. Errors quoted as ±95% confidence intervals. Measurements carried out at 32°C.

* (a) and (b) refer to duplicate specimens, † n = 5.

TABLE 5. Regeneration of stripped skin, *in vitro*, as measured by its permeability to tritiated water

Time (days)	Stripped skin in buffered isotonic saline pH 7·0	Stripped skin 1st day in saline, then growth medium	Stripped skin in growth medium		Unstripped skin in growth medium
			(a)*	(b)*	
	(n = 5)	(n = 5)	(n = 5)	(n = 5)	(n = 6)
0	137·7±13	124·3±4·7	126·6±14	103·7±15	11·6±2·8
1	95·7±23	70·7±15	80·3±10	53·4±7·4	12·7±1·1
2	87·5±21	57·6±7·1	64·5±6·3	45·3±3·2	13·2±2·1
3	91·8±19	50·1±4·5	50·7±4·9	38·9±6·0	12·2±1·1
4	97·6±12	45·2±5·7	45·3±6·0	35·1±5·3	12·5±2·8
8	101·4±16	34·6±4·4	36·2±4·5	29·2±2·7	12·2±2·6
11	103·5±18	30·6±6·1	31·4±4·7	27·1±3·2	14·0±1·2

Permeability coefficients (P) given in cm/s × 10^7. n = number of samples taken to calculate each permeability coefficient. Errors quoted as ±95% confidence limits. Measurements carried out at 37°C.

* (a) and (b) refer to duplicate specimens.

TABLE 6. Regeneration of stripped skin *in vitro* as measured by its permeability to tritiated water

Time (days)	Stripped skin in buffered isotonic saline pH 7·0	Stripped skin in growth medium (a)*	Stripped skin in growth medium (b)*	Stripped skin in growth medium (c)*	Unstripped skin in growth medium
	(n = 3)	(n = 6)	(n = 6)	(n = 6)	(n = 6)
1	308·3 ± 17·5	86·9 ± 12·7	172·8 ± 83†	27·1 ± 4·2	4·15 ± 0·43
3	330·0 ± 70	32·1 ± 9·2	70·4 ± 41·6	11·7 ± 2 6	4·09 ± 0·58
5	364·0 ± 95	38·9 ± 4·3	36·5 ± 7·8	14·1 ± 3·1	4·17 ± 0·5
7	278·0 ± 88	42·8 ± 7·2	68·2 ± 12·6	11·9 ± 2·2	4·08 ± 0·69
10	295·0 ± 6·6	35·8 ± 6·1	—	12·8 ± 0·6	4·77 ± 0·49
12	—	29·1 ± 10·6	—	12·1 ± 2·6	4·45 ± 0·93
14	—	31·2 ± 3·4	—	10·4 ± 9·4	3·32 ± 0·43
17	—	31·1 ± 5·4	—	9·2 ± 1·9	4·46 ± 0·34
21	—	36·8 ± 7·1	—	12·7 ± 2·4	5·16 ± 0·64
25	—	34·3 ± 8·4	—	14·3 ± 0 6	5·51 ± 0·99

Permeability coefficients (P) given in cm/s × 10^7. n = number of samples taken to calculate each permeability coefficient. Errors quoted as ±95% confidence limits. Measurements carried out at 32°C.

* (a), (b) and (c) refer to triplicate specimens, † n = 5.

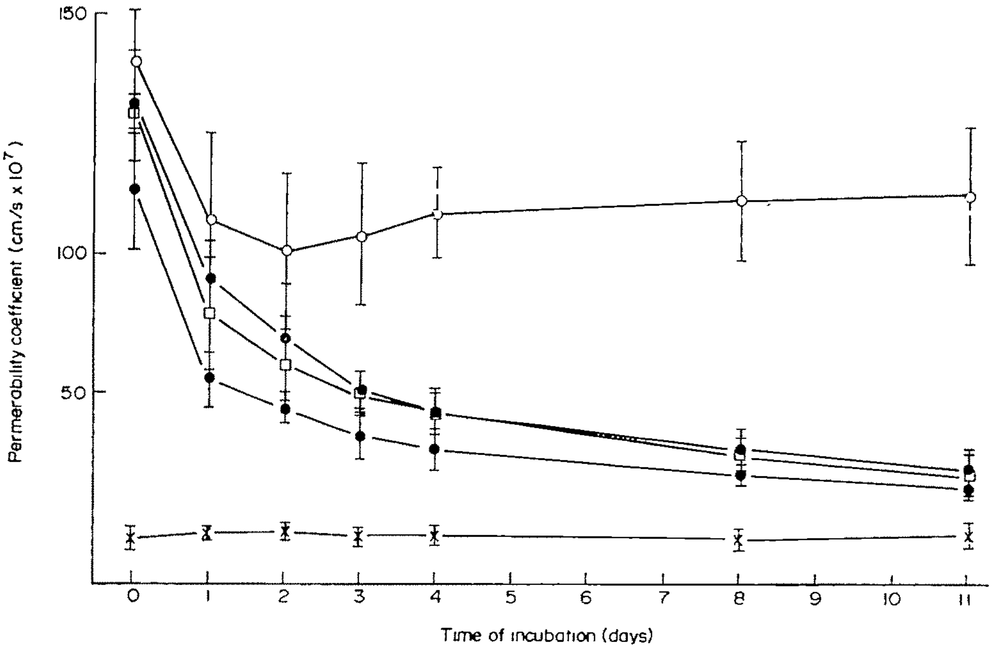

FIGURE 3. Effect of growth *in vitro* upon permeability of stripped human skin. ●, stripped specimens in growth medium; □, stripped specimen in saline for the first few hours, thereafter in growth medium; ○, stripped specimen in buffered saline; ×, unstripped specimen in growth medium. Stripped specimens in growth medium show appreciable regeneration over the whole period of observation.

An appreciable regeneration of barrier is observed in all specimens maintained in growth medium, the more complete the initial stripping the greater the proportionate decrease in permeability. Different specimens do not reach the same permeability, however, higher final values correlating with greater initial stripping. From Fig. 3 the regeneration is seen to proceed as an initial rapid decrease in permeability in the first 3–4 days, followed by a more gradual decrease.

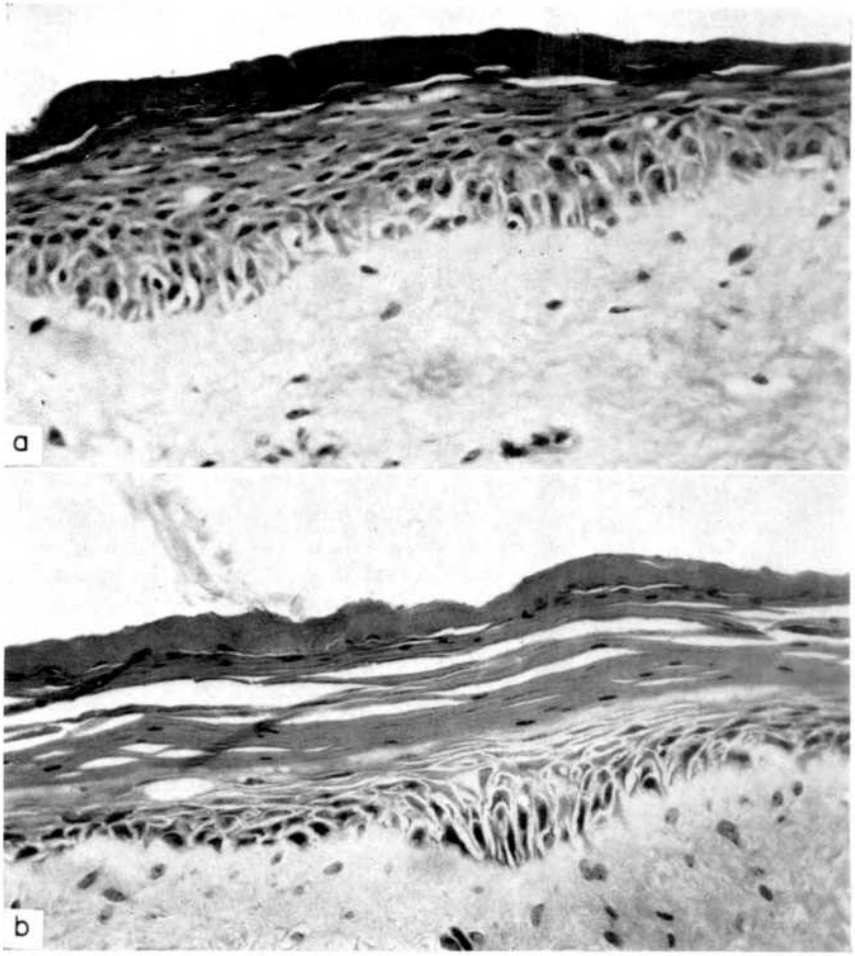

FIGURE 4. Stripped skin harvested after an incubation period of: (a) 4 days, (b) 11 days. Note the condensed, high-density layer at the surface in contrast to the open, basket-weave appearance of the stratum corneum of unstripped skin (H & E, × 256).

Histological examination of specimens maintained on nylon mesh supports in petri dishes indicates that parakeratotic tissue formation has two distinct phases. An outer dense layer is formed in the first few days of culture (Fig. 4a) and is presumably responsible for the sharp initial decrease in permeability. An inner expanded layer is then formed (Fig. 4b), which increases in thickness with time of culture but exerts a smaller effect upon permeability.

Autoradiographs prepared after exposure to ^{3}H-thymidine demonstrated an increased uptake into the basal and suprabasal cells of stripped skin, compared with unstripped specimens. Fig. 5 indicates how the number of labelled cells per section (3 mm length, 4 μm thick) varied over a period of 11 days in culture. Both stripped and unstripped skin are seen to follow a similar pattern of DNA synthesis *in vitro* when cultivated under identical conditions. There is an initial drop followed by a sharp increase between 3 and 5 days. The partially synchronous wave occurs in both cases.

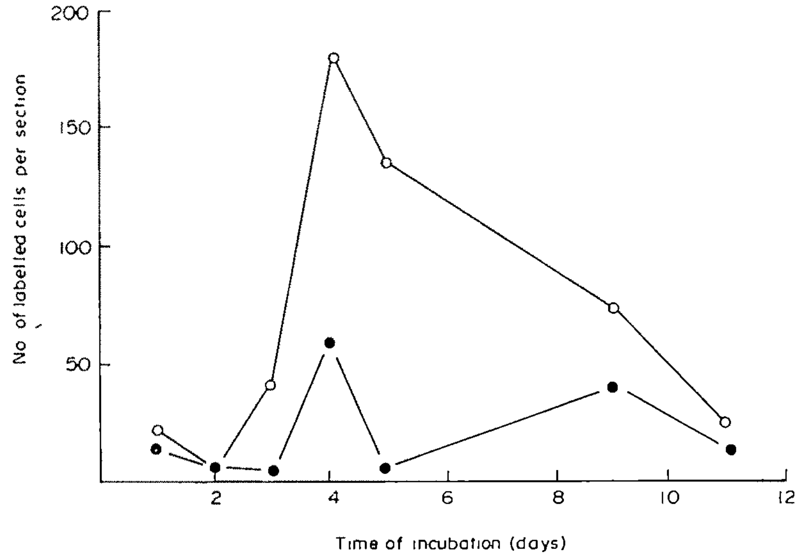

FIGURE 5. Incorporation of ^{3}H-thymidine into stripped and unstripped skin. Ordinate, number of labelled basal and suprabasal cells per section (4 mm length, 4 μm thick). 10 sections of each specimen were counted. Abscissa, number of days of incubation in MEM plus 10% human serum. A peak of DNA synthesis is observed at 4 days in both stripped and unstripped skin, the stripped specimens showing an enhanced incorporation over the whole period of culture. ○, stripped specimens; ●, unstripped specimens.

TABLE 7. A comparison of *in vivo* water vapour loss with *in vitro* permeability measurements

	In vitro	*In vivo*		
	Astley & Levine (present work)	Monash & Blank (1958)	Matoltsy *et al.* (1962)	Spruit & Malten (1966)
Before stripping	3·8 → 11·6	17	0·56	1·4
After stripping	29·3 → 308·3	78	28	125
3 days' growth	11·7 → 91·8	22	2·8	7·0

Permeability constants estimated from water vapour loss data assuming an effective water concentration gradient of 1 g/cm^3. Values given $cm/s \times 10^7$.

Throughout the whole period of incubation, DNA synthesis was higher in stripped explants. By the time the first wave of DNA synthesis occurs, barrier regeneration is nearly complete. The sharp rise in proliferation of the basal cell layer appears to follow the depletion of the Malpighian cells, which have been incorporated into the parakeratotic tissue.

No data have so far been reported for the *in vitro* regeneration of barrier function in human skin. There are, however, reports concerning *in vivo* regeneration. Malkinson (1958), measuring loss of ^{14}C-testosterone from the surface of stripped skin, reported complete renewal in 72 h. The technique is of limited accuracy, however, and the extent of regeneration in that time is uncertain. Malkinson comments on the difficulty encountered in assessing the end point of the stripping procedure.

Monash & Blank (1958), using water vapour loss as a measure of permeability, have followed barrier regeneration after removal of stratum corneum. A four- to five-fold increase in permeability was found after stripping. In the subsequent 2–3 days a rapid decrease in permeability was observed, which was associated with the production of a parakeratotic layer. This phase of regeneration was followed by a slow return to normal stratum corneum structure.

Matoltsy *et al.* (1962), also measuring water vapour loss, observed a fifty-fold increase in permeability upon stripping. In the first 3 days a ten-fold decrease occurred. During this period a temporary barrier is formed by rapid conversion of granular cells into parakeratotic tissue, a process very similar to that observed *in vitro*. The initial rapid fall in permeability is again followed by a much slower decrease, during which the skin structure returns to normal. This is not fully complete 8 days after stripping.

Spruit & Malten (1966) observed a ninety-fold increase in permeability upon stripping the skin. A rapid decrease in permeability occurred in the following 4 days, after which the barrier slowly returned to normal, the process being complete 30–40 days after stripping.

We have estimated permeability constants from data on evaporation, assuming an effective concentration gradient of 1 g/cm^3 across the stratum corneum (Tregear, 1966). These can be compared with our *in vitro* results (Table 7). Higher values are to be expected *in vitro*, since immersion of the specimens results in increased hydration of the stratum corneum. Taking this into account, the *in vitro* measurements are in fair agreement with the *in vivo* results.

Although a substantial decrease in permeability occurs *in vitro* during the rapid regeneration phase, the effect is more pronounced *in vivo*. The permeabilities attained at the end of this phase are, however, comparable in magnitude. The second phase, the return to normal observed *in vivo*, does not occur *in vitro*. Thus, although a more efficient and complete repair of barrier loss is observed *in vivo*, similar changes seem to occur *in vitro*, providing an extremely useful model for the study of skin behaviour. Assessment of damage or protection from materials applied either to the epidermal or the dermal surface, and of the effect of metabolic precursors or inhibitors, can be followed by permeability measurements as well as by changes in morphology and incorporation of labelled precursors.

APPENDIX I

From Fick's Law and the conservation of mass.

$$\mathrm{Vi}\,\frac{\mathrm{dCi}}{\mathrm{dt}} = \mathrm{P.A.}\,\{\mathrm{C_T} - \mathrm{Ci}\,[1 + (\mathrm{Vi/Vo})]\} \qquad \mathrm{I}(1)$$

Ci = concentration of penetrant below the skin, C_T = concentration of penetrant applied to the skin at time zero, Vi = volume of the chamber below the skin, Vo = volume of penetrant applied to the skin, P = permeability coefficient, A = area of skin exposed to penetrant.

When the first sample is removed $t = t^1$, and $\mathrm{Ci} = \mathrm{Ci}^1$. Integration of the above equation between the limits $\mathrm{Ci} = 0$ to $\mathrm{Ci} = \mathrm{Ci}^1$ and $t = 0$ to $t = t^1$ gives,

$$\frac{-\mathrm{Vi}}{(1 + \mathrm{Vi/Vo})} \ln \frac{\mathrm{C_T} - \mathrm{Ci}^1[1 + (\mathrm{Vi/Vo})]}{\mathrm{C_T}} = \mathrm{P.A.t}^1. \qquad \mathrm{I}(2)$$

When the second sample (x ml) is taken, a correction is necessary due to removal of the previous sample from the system, and integration is carried out between the limits $Ci = Ci^1 (1 - x/Vi)$ to $Ci = C^2$, and $t = t^1$ to $t = t^2$. This yields the following equation,

$$\frac{-VoVi}{Vo+Vi} \ln\left[\frac{C_T-(x/Vo)Ci^1-Ci^2\,[1+(Vi/Vo)]}{C_T-(x/Vo)\,Ci^1-Ci^1[1-(x/Vi)]\,[1+(Vi/Vo)]}\right] = P.A.\,(t^2-t^1) \qquad I(3)$$

Or, in the general case, where (n) refers to the number of the sample being taken

$$\frac{-VoVi}{Vi+Vo} \ln\left[\frac{C_T-(x/Vo)\,.\,\sum\limits_{[1\rightarrow(n-1)]} Ci)-Ci^n\,[1+(Vi/Vo)]}{C_T-(x/Vo\,.\,\sum\limits_{[1\rightarrow(n-1)]} Ci)-Ci^n\,[1-(x/Vi)]\,[1+(Vi/Vo)]}\right] = P.A.\,(t^{(n)}-t^{(n-1)}) \qquad I(4)$$

Permeability coefficients can be obtained either by calculation of a value for each sample taken, or by plotting the left hand side of equation I (4) *vs* $(t^{(n)} - t^{(n-1)})$ and calculating a value from the gradient of the best straight line. This latter method requires increasing time intervals between sampling to give a range of values for $(t^{(n)} - t^{(n-1)})$.

APPENDIX II

Calculation of permeability coefficient of parakeratotic tissue in unstripped skin

After incubation of unstripped skin for a few days, two distinct barrier layers are present, the original stratum corneum and the newly-formed parakeratotic tissue. The permeability coefficient (P) of the total barrier is related to the permeability coefficient (P_1) of the original stratum corneum, and the new parakeratotic layer (P_2) by the relation

$$\frac{1}{P} = \frac{1}{P_1} + \frac{1}{P_2} \qquad II(1)$$

P is the measured permeability coefficient of the cultured skin. A measure of P_1, the permeability coefficient of the original stratum corneum at time (t), can be obtained from the results of the specimen maintained in buffered saline, where the increase in permeability observed is due to alteration in barrier capacity of the original stratum corneum *only*.

Suppose $P_1(0)$ and $P_1(t)$ are the permeability coefficients of the original stratum corneum at time zero and (t) respectively, of specimens in growth medium. Ps(0) and Ps(t) are the permeability coefficients of the stratum corneum at time zero and (t) respectively, of the specimen maintained in buffered saline.

An estimate of $P_1(t)$ is obtained from

$$P_1(t) = P_1(0) \times Ps(t)/Ps(0) \qquad II(2)$$

P and P_1 are therefore known and the permeability coefficient (P_2) of the parakeratotic layer obtained from equation II(1). The values for P_2 from Table 1 are calculated and given in Tables 2 and 3. Several of the values of P_2 obtained at short incubation times have been ignored, since they are either negative or very small. The negative values result because calculation involves the term $PP_1/(P-P_1)$. At short incubation times P and P_1 are very similar and each is subject to an error. The difference $(P-P_1)$ is therefore very small, but subject to a large error, hence the resulting negative values. As the difference between P and P_1 increases the error in the difference is reduced and subsequent values give a reasonable indication of the barrier capacity.

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Ringworm in the South-West of England, 1960–1970, with special reference to onychomycosis

MARY P.ENGLISH AND LINDA LEWIS

Pathology Department, Royal Infirmary, Bristol BS2 8HW

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SUMMARY

The numbers of dermatophytes isolated from specimens sent to a mycological diagnostic service in the West of England from 1960 to 1970 are reported.

An increasing rate of failure to culture from microscopically positive specimens is noted. One reason for this was the large number of nail clippings sent in unaccompanied by scrapings from skin lesions. The cultural failure rate from nail clippings makes the diagnosis of the causal fungi in onychomycosis very unsatisfactory. For treatment purposes, means of deducing the nature of these uncultured fungi by combining clinical and laboratory observations are proposed.

It is suggested that two features of mycological diagnostic services—(a) failure to culture from a proportion of microscopically positive specimens, and (b) the fact that only the more severe, widespread and recalcitrant lesions are usually seen by dermatologists—result in a very misleading picture if these figures are used to assess the incidence of the various dermatophyte species in the general population.

A mycological diagnostic service centred on Bristol has been in existence since 1954. Over the years it has received dermatological specimens from an increasing area outside the city until, for example in 1970, nearly 3000 specimens were examined. The service is used for two main purposes: to identify the causal fungus in a given case of ringworm; and to confirm or exclude the presence of ringworm in lesions of doubtful aetiology. Because of the second usage, about two-thirds of all specimens examined are negative for fungi. Aspects of the findings during the first 6 years of the existence of the service (1954–1959) have already been reported (English, 1961); the present article covers the years 1960–1970 inclusive and is concerned solely with dermatophytes.

SOURCES OF MATERIAL

Bristol General Hospital

In the dermatological clinic of this hospital, scrapings were taken from all patients in whom a diagnosis of ringworm was a possibility. Consequently, its figures give an accurate picture of the true incidence of the various types of ringworm in dermatological patients.

Other sources

These are listed in Table 1 and include specimens sent by a number of general practitioners in the areas as well as from dermatological clinics at hospitals. Almost all the specimens are received by post. The doctors using the service have different interests, and therefore different criteria for requesting a mycological examination. The results from these centres may not, therefore, reflect accurately the true incidence of the various dermatophytes in clinic patients. For this reason they were considered separately from those for the Bristol General Hospital and the two sets of figures were combined only when they showed the same overall picture.

TABLE 1. Districts using the Bristol Mycological Diagnostic Service

District	Year in which service first used
Bristol General Hospital Other Bristol sources Weston-Super-Mare West Cornwall	before 1960
Gloucester and district	1961
Exeter and district	1962
Salisbury and district Swindon and district	1963
Oxford	1965
Neath and district	1966

METHODS

Direct microscopic examination in potassium hydroxide was carried out on all specimens. Scrapings were then inoculated on glucose–peptone agar with added chloramphenicol and actidione. When cultures from microscopically positive specimens inoculated on this medium failed, further cultures were made on malt agar if enough material remained. All microscopically positive nail clippings, as well as being submitted to the above procedure, were cultured on actidione-free medium, as onychomycosis may be caused by non-dermatophytes, indistinguishable from dermatophytes on direct microscopy, and sensitive to actidione. When two different fungi were found on the same patient, each is recorded separately.

RESULTS

Table 2 shows the total number of patients from whom dermatophytes were isolated in each of the 11 years. This number rose as the catchment area widened (see Table 1), then fluctuated at around 400 a year when the area using the service became constant in 1966.

Table 3 gives the total number of each dermatophyte isolated over the 11-year period. The fungi are grouped according to whether they are primarily human or animal parasites, or soil saprophytes. Figures for the Bristol General Hospital and 'other sources' are combined, as no differences were apparent.

Failed cultures

Table 3 does not give a true picture of the incidence of dermatophytes in dermatological patients, but only of those successfully cultured. Table 4 shows, separately for each year and for each source of specimens, the number of patients in whom microscopy for filamentous fungi was positive but cultures failed. Failed yeast cultures are excluded because yeasts, even in the mycelial phase, are distinguishable from the filamentous fungi on direct microscopy: many nail infections caused by moulds must, however, be included, as only those few that spore *in vivo* are distinguishable from dermatophytes in the absence of cultures. The years 1960–1964 inclusive are averaged, as the proportion of failed cultures remained fairly constant over this period. The gradual rise in this proportion in succeeding

TABLE 2. Number of patients per year from whom dermatophytes were isolated

Year	No. of patients	Year	No. of patients
1960	123	1966	422
1961	152	1967	382
1962	148	1968	443
1963	321	1969	411
1964	367	1970	365
1965	385		

TABLE 3. Incidence of dermatophytes from all sources

Type/Species	No.	% grand total
Anthropophilic		
M. audouini	44	1·6
T. rubrum	1492	42·3
T. interdigitale	576	16·3
T. tonsurans	34	1·0
T. violaceum	13	0·4
T. soudanense	4	0·1
E. floccosum	414	11·7
Total anthropophilic	2580	73·9
Zoophilic		
M. canis	165	4·9
M. persicolor	16	0·5
T. mentagrophytes	121	3·4
T. erinacei	64	1 8
T. equinum	4	0·1
T. quinckeanum	1	0·0
T. verrucosum	561	15·9
Total zoophilic	932	26·5
Geophilic		
M. gypseum complex	9	0·3
Grand total	3521	

years, especially from 'other sources', is evident. If Tables 2 and 4 are scrutinised together, it is obvious that as the proportion of failed cultures rises Table 2 gives an increasingly inaccurate assessment of the incidence of dermatophytes over the 11-year period. Moreover, unless these failed cultures are evenly distributed amongst the dermatophyte species, the apparent relative incidence of the species must be distorted. A failure rate of 22% (other sources, 1970) is likely to produce a gross misrepresentation of the true relative incidence of the species. The inaccuracies introduced into Table 2 by failure of cultures must be taken into account during the following discussion of the individual dermatophyte species.

TABLE 4. Annual record of failed cultures

Year	Patients from Bristol General Hospital			Patients from other sources		
	No. of failed cultures	Total microscopically positive	% failed cultures	No. of failed cultures	Total microscopically positive	% failed cultures
1960–1964 (averaged)	10	93	11	38	277	14
1965	13	134	10	63	526	12
1966	16	138	12	89	702	13
1967	8	114	7	80	550	14
1968	13	127	10	105	689	15
1969	16	111	14	118	681	17
1970	19	111	17	136	632	22

TABLE 5. Incidence of *T. rubrum* as % of '*T. rubrum** + *T. interdigitale*†' and as % of 'total dermatophytes' over the period of the survey

Year	From all sites					
	Bristol General Hospital			All sources		
	Total *Tr*	*Tr* as % of *Tr* + *Ti*	*Tr* as % of total dermatophytes	Total *Tr*	*Tr* as % of *Tr* + *Ti*	*Tr* as % of total dermatophytes
1960–1962	53	No significant change		95	No significant change	Significant change ($P > 0{\cdot}001$)
1963	19			77		
1964–1970	204			1002		
	From feet only					
1960–1962	37	No significant change		48	No significant change	
1963	14			34		
1964–1970	125			623		

* *Tr*, *T. rubrum*; † *Ti*, *T. interdigitale*

Anthropophilic species

Microsporum audouinii. Cases of scalp ringworm due to this fungus were scattered over the area, except for one outbreak of eight linked infections in Bristol in 1967–1968.

Trichophyton rubrum and *Trichophyton interdigitale.* Table 3 shows that *T. rubrum* is the most common of all the dermatophyte species isolated, and that it was found about 2½ times more often than *T. interdigitale*, the next most common anthropophilic species. These figures are in agreement with those of diagnostic services in Europe and North America. These two species account for the vast majority of cases of tinea pedis and toe-nail infections seen in dermatological clinics. Since the incidence of *T. rubrum* has been increasing in developed countries since the First World War, our figures for this fungus were analyzed in various ways in an attempt to determine if this trend is still continuing (Table 5). Because the total number of isolations per year changed considerably during the 11-year period under study (Table 2), the period was divided into three sub-periods (1960–1962, 1963 and 1964–1970), in each of which the total number of fungal isolations per year was approximately the same. The total incidence of *T. rubrum* in these periods, and its incidence in tinea pedis alone, both for the Bristol General Hospital and for 'other sources' were calculated as a percentage of all dermatophytes isolated and of '*T. rubrum* + *T. interdigitale*', and the χ^2 test applied to determine if there was any significant rise in the incidence of *T. rubrum*. Table 5 shows that of the eight combinations, only the number of *T. rubrum* isolations from 'other sources', expressed as a percentage of the total dermatophyte isolation, rose significantly over the period. As there was no corresponding rise in the figures for the Bristol General Hospital, it is possible that this could indicate merely that certain dermatologists outside Bristol were taking a special interest in *T. rubrum*.

Trichophyton soudanense. Three patients were Africans, and two of these contracted their infections in their own countries. The third was a child born in Britain, who had never been to Africa. None of her siblings was infected; unfortunately it was not possible to examine her parents. A fourth patient was an English nurse who had spent a holiday in Ghana and was aware that she had been in contact with an infected Ghanaian child.

Trichophyton schoenleinii. The six patients were British and only two were related. The origins of the infections were not discovered.

Zoophilic species

Microsporum canis. There were four outbreaks of infection by this fungus, in each of which the cases were epidemiologically linked. One outbreak (seven cases) occurred in Bristol in 1963, one (four cases) in the Gloucester district in 1965, one (five cases) in the Salisbury district in the same year, and one (fourteen cases) in West Cornwall in 1970.

Microsporum persicolor, the agent of vole ringworm, is very common in voles but rare in man. Of the sixteen isolations from humans made over the period, nine occurred in 1964. This may reflect the tendency, well known to zoologists, for violent fluctuations in the vole population from year to year.

Trichophyton erinacei. This fungus was not recognized as distinct from *T. mentagrophytes* until 1963 (Smith & Marples, 1963), and was not recorded separately from that fungus during the early years of the present survey. The maximum number of human cases in any year was twelve (in 1968). Some cases were shown to be direct infections from a hedgehog; others were indirectly contracted from hedgehog-hunting dogs or from hedgehog nesting sites or other contaminated, inanimate objects.

Trichophyton verrucosum. This is the most commonly encountered zoophilic fungus in the South-West. It equals *T. interdigitale* in being the second most common of all dermatophyte species from specimens sent in by local doctors (Table 3). Even so, it is undoubtedly under-represented in these figures. Both patients and general practitioners from farming areas are very familiar with 'cattle ringworm' and large numbers of patients never reach the dermatology clinics. It is probably for this reason that, though its incidence was higher in winter and lower in summer when averaged over the 11 years, there was no definite winter peak or summer trough, such as is found when every member of an exposed community is examined (Chmel, 1967).

Failed cultures: the special case of nail clippings

One of the many reasons for failure of growth from microscopically positive specimens (English, 1961) was investigated in detail. The difficulty of culturing from nail clippings is well known and arises because, in most cases of onychomycosis, the active growth of the fungus takes place in the proximal part of the nail; very often the hyphae are dead by the time they have been carried by nail growth to the distal end from which the clipping is taken (Gentles, 1971). If scrapings from any associated skin lesions yield a dermatophyte it is probable (though not inevitable) that the same fungus is causing the nail lesions. There may be no associated skin lesions, or scrapings from such as exist may not be sent to the laboratory; in such cases there is no clue as to the fungus causing the onychomycosis. Table 6 shows the increased incidence of 'nail only' specimens sent for diagnosis in

TABLE 6. Nail clippings as a factor affecting the overall rate of cultural failures. Comparison of 1965 and 1970 for specimens from all sources

		Cultures failed				
		Total cases		Only nail clippings sent (cases)		
Year	Total microscopically positive cases	No.	% microscopically positive cases	No.	% microscopically positive cases	% total 'cultures failed'
1965	660	76	11·5	39	6	51
1970	743	155	20·9	74	10	48

TABLE 7. Infected nails without accompanying mycotic skin lesions as a proportion of of total failed cultures

	Failed cultures		
Year	Total	Infected nails without mycotic skin lesions	% nails
1970	155	74	48
1972	187	80	43

1965 and 1970, when the total number of failed cultures was rising. The percentage of successful isolations from nails, though low, remained practically constant over this period and was not, therefore, a factor in the increasing proportion of failed cultures.

It seemed possible that this increase in the number of 'nail only' specimens might reflect an increasing interest on the part of dermatologists in onychomycosis without associated skin lesions. Therefore, in 1971 and 1972, clinicians were asked to send in skin scrapings with nail clippings whenever this was possible. The response to this request was good, but many of the skin scrapings were free from fungi, thus tending to confirm the above hypothesis. That the interest of clinicians in onychomycosis without associated skin lesions continued at approximately the same level from 1970 to 1972, is shown in Table 7. Table 8, showing that the overall percentage of failed cultures also levelled out over this period, would appear to confirm this. That the peak in the number of 'nail only' specimens was reached in 1970 could have been either because dermatologists were already taking clippings from most patients presenting with onchomycosis, or they may have been discouraged from taking more clippings by the very high rate of cultural failures.

TABLE 8. Percentage of failed cultures, 1970–1972

Year	Total microscopically positive cases	Failed cultures	
		No.	%
1970	743	155	20·9
1971	695	127	18·2
1972	854	163	19·2

DISCUSSION

An important point arising from this study is that figures from diagnostic services (which, in practice, are those from dermatological clinics) in no way represent either the actual or relative incidence of the individual dermatophyte species in the general population. This is not generally taken into account in reports published from diagnostic services. There are two main reasons for the discrepancy.

First, usually only the more severe, recalcitrant or diagnostically difficult ringworm lesions ever reach dermatological clinics: the rest are treated by the patient himself or by his general practitioner. This is especially true of tinea pedis due to *T. interdigitale* which, in contrast to the impression given by the diagnostic service figures, is far more common in the general population in Britain than is tinea pedis caused by *T. rubrum* (English, 1969; Gentles & Evans, 1973). All other species causing mild or clinically easily diagnosed lesions, including *M. persicolor*, *T. equinum*, and *T. verrucosum*, are grossly under-represented in figures from diagnostic services serving mainly dermatologists.

Though our figures show no very convincing increase of *T. rubrum* over *T. interdigitale* over the 11 years, such figures would be strongly influenced by our increasing incidence of failed cultures, and hence little reliance should be placed on them.

The second cause for suspicion of figures from diagnostic services is the number of failures to culture a fungus which has been seen on microscopy. A failure rate of 10% is not unusual in any service; some of the reasons for it have been discussed previously (English, 1961). It is now known that the addition of actidione to the medium may occasionally inhibit a dermatophyte as well as contaminant moulds. Re-culturing on actidione-free medium can be carried out only if enough of the original specimen remains.

In this study the level of 'failed cultures' is consistently higher in specimens from 'other sources' than in those from the Bristol General Hospital. There are two possible reasons for this. The delay caused by posting the specimen from outside Bristol might affect the viability of the fungus; but there is good evidence (Dvořák & Hubálek, 1968; English, unpublished) that, for up to a week at least, this does not affect the success of dermatophyte cultures provided the specimens are kept dry. It would seem more likely that specimens from the Bristol General Hospital, being taken by a limited number of trained persons, are in general more satisfactory than those from 'other sources' which are taken by a great variety of persons, many of whom may have little knowledge of the type of specimen needed.

However, the most disturbing feature of this study is the rapid increase in both series of patients in the number of failed cultures since 1965. One possible contributory factor is the increased use of topical corticosteroids on lesions of unknown aetiology by general practitioners. This has two effects: it greatly assists direct microscopy, rendering any fungus present very easily visible; but it also reduces scaling, and scrapings obtained from steroid-treated lesions are often inadequate for proper laboratory investigation. This may account for the fact that, in 1970, cultures from seventeen microscopically positive groin lesions, all recently treated with steroids, failed to grow, though this site is usually an easy one from which to obtain cultures. Possibly the Sellotape stripping technique (Knudsen, 1971) would be useful in these cases.

TABLE 9. Key for diagnosis of nail infections due to filamentous fungi of which cultures have failed

Nail(s) infected	Accompanying features	Probable fungus in the nail lesion
Finger and toe	Fungal skin lesions anywhere on the body, especially the feet	*T. rubrum*
Toe only	(1) Tinea pedis	(1) Same dermatophyte as that causing the tinea pedis
	(2) No skin lesions on feet, or lesions of non-mycotic origin	(2) *T. interdigitale* or a non-dermatophyte
Finger only	No mycotic lesion of skin	Non-dermatophyte

The reason singled out for special study, was, however, the low success rate of cultures from nails, coupled with the increasing number of nail clippings sent in without scrapings from associated skin lesions. This obvious interest of dermatologists in onychomycosis not associated with fungal lesions of the skin suggests that, until improved methods of obtaining nail specimens for culture are universally available, a reasonably accurate method of assessing the type of fungus present in a nail in which a fungus can be seen, but from which it cannot be cultured, would be helpful for treatment purposes. Walshe & English (1966) have shown that, in toe-nails, such infections are likely to be due to *T. interdigitale* or to non-dermatophytes. This has recently been confirmed by English & Atkinson (1973), who used a drill to obtain nail samples, attaining a cultural success rate of about 90%. Little work has been done on finger-nails, but where cultures have been successful, in cases where there are no mycotic lesions elsewhere on the body, a non-dermatophyte has usually been isolated.

The suggestions in Table 9 are therefore put forward as a reasonably reliable synthesis of laboratory and clinical observations for a temporary solution to this problem.

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Chronic *Candida albicans* paronychia in adult Israeli women

SOURCES AND SPREAD

SALOMON GANOR AND RAPHAEL PUMPIANSKI

Outpatient Department of Dermatology and Venereology and Outpatient Department of Obstetrics and Gynaecology, Kupath Holim, Jersualem, Israel

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SUMMARY

The infected fingers in chronic paronychia were mostly on the dominant hand. Little fingers were less and middle fingers more affected than expected. Oral *Candida albicans* carriage was significantly less when the first finger to be infected was the middle finger. The duration of the disease was related to the number of infected fingers, but not to oral *C. albicans* carriage. The second finger to be infected was an opposite* or adjacent finger more often than expected.

The following conclusions are drawn: (1) The mouth and the bowel, but not the vagina, of the patient, or her family, are sources of *C. albicans* in chronic paronychia. (2) Mechanical trauma that destroys the cuticle is responsible for the invasion of *C. albicans* into the nail-fold. (3) This trauma in our patients is mostly due to rubbing during hand-washing of clothes.

Chronic paronychia is a frequently encountered infection due to *Candida albicans* (Raubitschek, 1946; Hellier, 1955; Frain-Bell, 1957; Whittle, Moffat & Davis, 1959; Esteves, 1959; Marten, 1959; Stone & Mullins, 1963), and rarely to other Candida species. It is much more prevalent in females than in males (Hellier, 1955; Frain-Bell, 1957). In addition the middle finger of the right hand is more often affected than other fingers (Frain-Bell, 1957; Cordero, Woscoff & Medeo, 1970; Barlow *et al.*, 1970). Whittle *et al.* (1959) pointed out that, in their studies, the first finger to be affected was the middle finger of the right hand.

A previous study (Ganor & Pumpianski, 1970) revealed that *C. albicans* was more often found in the mouth of female patients with chronic paronychia than in control females matched for age. However, Cordero *et al.* (1970) found no connection between the presence of *C. albicans* in the mouth and chronic paronychia.

Stone & Mullins (1964) were able to induce chronic paronychia experimentally by inoculating *C. albicans* into the nail-fold, and then occluding the nail with adhesive tape for 30 days. In this context, an editorial in the *Lancet* (1970) pointed out: 'in the naturally occurring disease there is no prolonged occlusion to open up the nail-fold, and some other mechanism must be at work'.

Reprint requests to Dr. S. Ganor, Outpatient Department of Dermatology and Venereology, Kupat Holim, 26 Ben Yehudah Street, Jerusalem, Israel.

* The term opposite finger, is used for the corresponding finger on the other hand.

This study, involving only adult females, was undertaken in order to obtain better insight into those mechanisms which instigate chronic paronychia. Its aims were; (1) to determine if a relationship exists between the presence of *C. albicans* in the oral cavity and the first finger to be infected; and (2) to ascertain the method by which subsequent fingers became infected.

MATERIALS AND METHODS

130 women over 16 years of age, who suffered from chronic paronychia, were examined. Their hands were classified as 'most used hand' (MUH) and 'least used hand' (LUH) to correct for left handedness. Swabs for *C. albicans* were taken from the patients' mouths and inoculated directly onto Sabouraud's dextrose agar and taurocholate agar. *C. albicans* was identified by the development of chlamydospores on taurocholate agar (Bakerspigel, 1962; Evron & Ganor, 1968), or, in the absence of chlamydospores, by the development of pseudo germ tubes in human serum at 37°C (Taschdjian, Burchall & Kozinn, 1960). The date of the onset of the infection was noted, as well as the order in which the fingers became infected. The first finger infected was classified as FFI, the second finger to be infected SFI, etc. Candida species other than *C. albicans* were isolated from the mouths of six patients in the series; these were excluded from this study.

TABLE 1. Relationship between oral *Candida albicans* carriage and first infected finger

	Thumb		Index f.		Middle f.		Ring f.		Little f.	
	MUH	LUH	MUH	LUH	MUH	LUH	MUH	LUH	MUH	LUH
C. albicans	13	6	11	8	6	3	4	2	2	1
No Candida	9	3	7	2	16	3	4	0	1	0
Total	22	9	18	10	22	6	8	2	3	1

TABLE 2. Duration of disease and oral *Candida albicans* carriage

	C. albicans	No Candida
Less than 1 year	23	26
More than 1 year	24	21
Total	47	47

RESULTS

The FFI was on the MUH in eighty-nine patients and on the LUH in forty-one patients. There was a significant difference between the MUH and LUH ($P<0.001$; $\chi^2=17{\cdot}0$). The thumb was the FFI in thirty-seven cases, the index finger in thirty-two, the middle finger in thirty-nine, the ring finger in seventeen, and the little finger in five. Although the total number of FFIs on each hand was different, the opposite fingers of the MUH and the LUH did not differ statistically from each other as a percentage of the total. Thus they were combined for statistical significance tests between the fingers. The difference between the fingers was significant ($P<0{\cdot}001$; $\chi^2=32{\cdot}6$). The order of deviation from expected was: the little finger had a much lower incidence of infection than expected, and both the middle finger and the thumb showed a much higher incidence than expected.

As shown in Table 1, *C. albicans* was seen to be present in the mouth of nineteen out of thirty-one patients when the thumb was the FFI. When the index finger was FFI nineteen out of twenty-eight carried *C. albicans* in the mouth, when it was the middle finger nine out of twenty-eight, when it was the ring finger six out of ten, and in the little finger, three out of four. There was a marked lowering of incidence of *C. albicans* carried in the mouth when the FFI was the middle finger than when the FFIs were other fingers ($P<0{\cdot}05$; $\chi^2=10{\cdot}4$; opponent fingers of MUH and LUH combined).

C. albicans was found in the mouth of twenty-three out of forty-nine patients with chronic paronychia of less than 1 year's duration, and in twenty-four out of forty-five patients who were infected for more than 1 year. As shown in Table 2, there was no statistical significance.

TABLE 3. Number of infected fingers and duration of the disease

	One finger	2–3 fingers	4 or more fingers
Less than 1 year	38	20	10
More than 1 year	13	16	21
Total	51	36	31

TABLE 4. Chronic paronychia: second infected finger

	Most used hand	Least used hand	Total
Thumb	3	5	8
Index finger	14	3	17
Middle finger	13	6	19
Ring finger	7	3	10
Little finger	1	1	2
Total	38	18	56

TABLE 5. Relationship of second infected finger to first infected finger

Opposite	17
Adjacent	21
Other	18
Total	56

Table 3 shows that the number of infected fingers is related to the duration of the infection. In the first year of infection one finger was affected in thirty-eight patients, more than one finger in thirty patients, while ten patients had four or more fingers affected. After the first year, one finger was affected in thirteen patients, and more than one finger in thirty-seven; of the latter, twenty-one had four or more fingers affected. This was a significant difference ($P<0{\cdot}001$; $\chi^2=15{\cdot}1$). The number of affected fingers was not related either to the presence of *C. albicans* in the mouth or to the FFI.

Table 4 shows the second finger to be infected (SFI). In thirty-eight patients the SFI was on the MUH, while it was on the LUH in eighteen patients. The difference between the hands is statistically significant ($P<0{\cdot}01$; $\chi^2=7{\cdot}1$). The thumb was the SFI eight times, the index finger seventeen times, the middle finger nineteen times, the ring finger ten times, and the little finger in two cases. Although the total number of SFIs of each hand was different, the opposite fingers of each hand did not differ statistically from each other as a percentage of the total.

Thus they were combined for statistical significance test between the different fingers; there is a statistical difference between them ($P<0{\cdot}01$; $\chi^\circ=17{\cdot}4$). The little finger was less often the SFI and the middle finger more often the SFI than expected.

Table 5 shows the relationship between the SFI and the FFI. In seventeen cases the SFI was the opposite finger of the FFI, the adjacent finger in twenty-one, and another finger in eighteen cases. If there had been equal chances for each finger to be the SFI, then the opposite finger of FFI would have been affected in five out of forty-five patients (six or seven out of fifty-six), the adjacent fingers in eight out of forty-five (nine or ten out of fifty-six) and thirty-two out of forty-five would have been another finger, neither opponent or adjacent (thirty-nine or forty out of fifty-six).

This means that the deviation from expected is very high, both for the opposite and the adjacent fingers, as compared to other fingers ($P<0{\cdot}001$; $\chi^2=44{\cdot}4$). The deviation from expected is far greater for the opposite finger than for the adjacent finger. This distribution pattern followed similar lines no matter which finger was the FFI.

The number of fingers infected thirdly and subsequently was too small for statistical evaluation.

DISCUSSION

Theoretically, the source of *C. albicans* in the FFI could be the vagina, mouth or bowel of the patient Another possible source might be contaminated family underwear or napkins hand-washed by the patient.

However, *C. albicans* is rarely found in the vagina of patients with chronic paronychia (Whittle *et al.*, 1959; Marten, 1959; Ganor & Pumpianski, 1970), and so this site seems to have little epidemiological significance. Other evidence that the vagina is not involved will be given later in the discussion.

It is assumed that the other three factors may each provide infective sources of *C. albicans*, although no firm conclusion can be drawn from this study as to whether either contact with the stool or the washing of contaminated underwear, or both, are epidemiologically important. Two facts, however, strengthen the assumption that the patient's mouth and stool (and/or the washing of underwear) are important sources of *C. albicans* infection in chronic paronychia.

(1) When the FFI was the middle finger *C. albicans* was present less often in the mouth than when other fingers were the first to be infected. Both the thumb and index fingers are in more intimate contact with the mouth than the middle finger while eating or removing food particles from between the teeth, or in handling dental prostheses.

(2) Frain-Bell (1957), Whittle *et al.* (1959), Marten (1959) and Barlow *et al.* (1970) found enterobacteriaceae and/or coagulase-positive staphylococci at the site of the paronychial infections in some of their patients. (It is not clear from their studies whether these bacteria were found alone or in combination.) Stone & Mullins (1962), however, found streptococci (on one occasion enterococci) and enterobacteriaceae in the paronychial site of their five biopsied patients. Since enterococci and enterobacteriaceae are primarily intestinal flora, and streptococci and coagulase-positive staphylococci are upper respiratory tract flora, it is possible that the intestinal flora is present more often when the source of *C. albicans* is the stool of the patient or her family, whereas the respiratory tract flora is found more often when the source of *C. albicans* is the mouth.

In this context, it is relevant to mention that vaginal bacterial flora have not been found in chronic paronychia, strengthening the conclusion that the vagina is not a source of infection in chronic paronychia. To our knowledge, no study has yet been done to correlate the presence of oral and faecal *C. albicans* in patients with chronic paronychia with the presence, or absence, in the paronychial site, of coagulase-positive staphylococci and streptococci on the one hand, and of enterobacteriaceae on the other.

Table 2 shows that there is no significant rise in the incidence of *C. albicans* found in the mouth when the chronic paronychia is of extended duration, obviating the possibility of the mouth being secondarily infected from paronychia, rather than the nail fold being infected from the mouth.

In a previous study (Ganor & Pumpianski, 1970), we found a positive correlation between the presence of *C. albicans* in the mouth and chronic paronychia. Cordero *et al.* (1970) did not make this same correlation. In our opinion a salient fact not covered in either publication was the numerical incidence of the FFI.

A theory which explains the pathogenesis of chronic paronychia in adult Israeli women must take into account the six facts emerging from this study; the first three emerging also from other studies (Frain-Bell, 1957; Whittle *et al.*, 1959; Barlow *et al.*, 1970):

(1) The MUH is more affected than the LUH, (2) the little fingers are much less often affected than expected, (3) the middle fingers are more frequently affected than expected, (4) the number of affected fingers rises with the duration of the infection, (5) the SFI is the finger opposite to the FFI more often than expected, and (6) the SFI is the finger adjacent to the FFI more often than expected.

One could not adequately explain these phenomena if spread of the paronychia was due only to finger contact with mouth or stool, or only to contact with water (Esteves, 1959; Wilson, 1965). Therefore, in accord with a theory already propounded (Whittle *et al.*, 1959), we postulate that two factors are necessary for the development of chronic paronychia: (1) contact with the source (mouth or stool of the patient or patient's family), this contact being made more often by the MUH than by the LUH, and only rarely by the little finger; (2) the occurrence of mechanical trauma which destroys the cuticle and facilitates infection of the nail-fold.

Although several types of trauma may be of significance (Whittle *et al.*, 1959; Esteves, 1959), it seems that the mechanical trauma experienced when hand-washing clothes is of primary importance, since it is the only trauma which explains why the number of infected fingers rises with the duration of the disease, and why the SFI is the opposite or adjacent finger to the FFI more often than expected.

Most patients wash their clothes by scrubbing them over the dorsa of the distal phalanges of the fingers, particularly the middle finger. The little finger is rarely used in the washing process. Although many of our patients have washing machines, they customarily do small washes by hand, and also hand-scrub the most soiled portions of the laundry before machine-washing it. Of ten patients who were specifically asked, only one (who cleaned her nails with a brush) did not rub the laundry over the dorsum of the proximal phalanx of the FFI.

Consistent with the hypothesis developed above is the fact that most patients with chronic paronychia are housewives or domestic workers (Hellier, 1955; Frain-Bell, 1957; Whittle *et al.*, 1959; Esteves, 1959; Marten, 1959; Stone & Mullins, 1963; Cordero *et al.*, 1970; Wilson, 1965). It is also consistent with an uncontrolled study showing that a nail-polish seal, together with anticandidal treatment, is effective in healing paronychia, when applied in the morning before work and removed, only at bedtime, with acetone (Garrard, 1955). This provides a barrier layer for the infected nail-folds acting in place of the cuticle which was disrupted by trauma.

Our hypothesis also explains the differing results of experimental infection. Stone & Mullins (1964) succeeded in establishing an acute *C. albicans* infection after occlusion of the nail-fold with adhesive tape for 30 days. However, at that time, neither rounding out nor retraction of the nail-fold had occurred; but they did occur 7–10 days after removal of the tape. Apparently, removal of the tape, which stripped the cuticle, was necessary for invasion of the nail-fold by *C. albicans*, which was already present in the environment. Other investigators who bandaged the nail-folds with cellophane which was then covered by adhesive tape (Vilanova, Casanovas & Francino, 1956), or who covered the nails with rubber cots (Maibach & Kligman, 1962), failed to induce chronic paronychia. But when the nail-fold was occluded with adhesive tape every 2–3 days (Whittle & Gresham, 1963), the posterior nail-fold was infected twice by *C. guillermondii* which was colonizing the nail-fold.

This hypothesis explains also why chronic paronychia rarely occurs in toes, although *C. albicans* is present very often on feet which perspire freely due to occupation, and where maceration is as often present as on the hands of housewives (Winner & Hurley, 1964).

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Pharmacology and Treatment

Evaluation of the efficacy of topical corticosteroids

S.T.ZAYNOUN AND A.K.KURBAN

Division of Dermatology, Department of Internal Medicine,
American University of Beirut Hospital, Beirut, Lebanon

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SUMMARY

The potency of topical corticosteroids has been evaluated using a recently described test which measures both their vasoconstrictor and anti-inflammatory activities. Betamethasone valerate, fluocinolone acetonide and triamcinolone acetonide creams were found to have practically the same potency. They were all superior to a combination of fluocortolone caproate and fluocortolone trimethylacetate cream. A remarkable difference was observed between the vasoconstrictor and the anti-inflammatory activity of the latter preparation. The significance of this finding is discussed.

The experimental evaluation of the potency of topically applied corticosteroids is difficult. The basis for such an evaluation has been either the ability to control induced inflammation or to produce dermal vasoconstriction. Several procedures have been described to test the anti-inflammatory effect of topical steroids. Inflammation can be produced experimentally by a variety of means, including electrophoretically induced eczematous reactions (Hexthausen, 1956); cellophane tape stripping (Wells, 1957); irradiation with ultraviolet light (Scott & Kalz, 1956); and application of mustard oil and nitric acid (Scott & Kalz, 1956), croton oil (Witkowski & Kligman, 1959) or tetrahydrofurfuryl alcohol (Schlagel & Northam, 1959; Schlagel, 1965, 1972; Brunner & Finkelstein, 1960). All these procedures have drawbacks, and a more promising approach was thought to be the investigation of the ability of potent corticosteroids to produce blanching when in contact with the skin (McKenzie & Stoughton, 1962; McKenzie, 1962; McKenzie & Atkinson, 1964; Stoughton, 1969; Child *et al.*, 1968; Place, Velazquez & Burdick, 1970). Moore-Robinson & Christie (1970) modified this assay system and evolved a method which permitted the simultaneous evaluation of blanching and the control of irritation such as that produced by croton oil.

Recently, Kaidbey & Kligman (1972) described a method using Ultracin (100% Kerosin) as a primary irritant following the topical application of different steroids under occlusion. By this method, the vasoconstrictor potency as well as the efficacy in suppressing experimentally-induced inflammation are assessed in the same experimental set-up. The purpose of this study is to use this test procedure to compare the efficacy of different commercially available topical corticosteroids and to find the degree of correlation between vasoconstriction and suppression of experimentally induced inflammation.

MATERIALS AND METHODS

For these investigations the following four preparations were used: betamethasone 17-valerate cream, 0·1% (Celestoderm-V, Schering Corporation, U.S.A.); fluocinolone acetonide cream, 0·025% (Synalar, Syntex Company, U.S.A.); triamcinolone acetonide cream, 0·1% (Kenacort-A cream, Squibb Company, U.S.A.); and a combination of fluocortolone caproate, 0·025%, and fluocortolone trimethylacetate, 0·025%, cream (Ultralan, Schering Company, Germany). Aqueous vanishing cream base was used as control. The steroid and the control creams were put separately in different coded tubes, and the nature of the cream used was not known to the investigators or the subjects.

To assess the vasoconstrictor potency of the creams, a modified McKenzie–Stoughton (1962) technique was employed. Groups of healthy male medical students were utilized. The experiments were started at 8.30 a.m. and lasted for 24 h. The skin over the lower back was cleaned with ether. Four squares, 1·3 × 1·3 cm each and 3 cm apart, were outlined on each side in the paravertebral areas using a rubber stamp. Liberal amounts of the creams were applied to cover the whole area of the corresponding squares. These were then covered with gauze and occluded with Blenderm Surgical Tape (3M Company). The surrounding skin was sprayed with Dow Corning Medical Adhesive B and the whole area covered with adhesive tape. 6 h later the occlusive dressings were removed and the sites examined independently for vasoconstriction under day light by two different examiners. The degrees of vasoconstriction were graded as follows: grade 0, no blanching; grade I, faint blanching; grade II, definite blanching.

The creams were then wiped off and the squares covered with 1·3 × 1·3 cm absorbent cotton pieces. Ultracin 0·1 ml was delivered to each cotton piece and the sites were occluded as described above, till the next morning at 8.30 a.m. The dressings were removed and the degrees of vesiculation were graded as follows:‡ grade 0, no vesicles; grade I, vesicles occupying 25% of area of square; grade II, vesicles occupying 50% of area of square; grade III, vesicles occupying 75% of area of square; grade IV, one bulla covering the square.

TABLE 1. The blanching and anti-inflammatory effects of topical corticosteroids

Drug	Blanching (grade)			Vesiculation (grade)				
	0	1	2	0	1	2	3	4
Betamethasone 17-valerate	3* (5)†	14 (25)	39 (70)	40 (72)	9 (16)	6 (10)	1 (2)	0 (0)
Fluocinolone acetonide	12 (25)	15 (31)	21 (44)	35 (73)	8 (17)	2 (4)	2 (4)	1 (2)
Triamcinolone acetonide	5 (17)	7 (24)	17 (59)	15 (51)	8 (28)	2 (7)	2 (7)	2 (7)
Fluocortolone caproate and trimethylacetate	0 (0)	10 (50)	10 (50)	1 (5)	4 (20)	2 (10)	7 (35)	6 (30)
Placebo	29 (71)	11 (27)	1 (2)	4 (10)	3 (7)	6 (15)	10 (24)	18 (44)

* No. of patients.

† Figures in brackets represent percentage of patients.

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‡ Visual estimation.

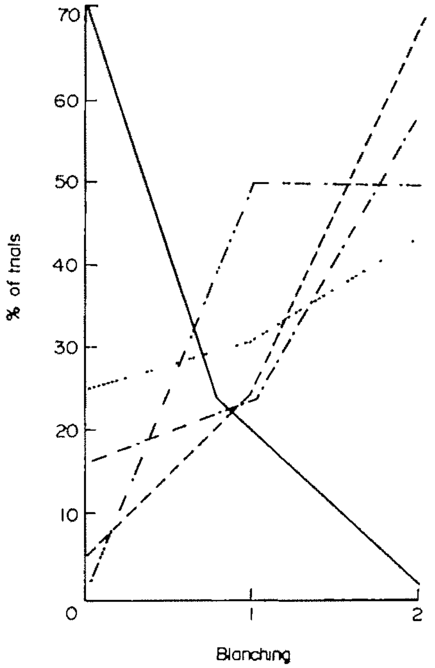

FIGURE 1. Blanching effects of topical corticosteroids. – – – –, betamethasone 17-valerate; —·—·—·, triamcinolone acetonide; —··—··—··, fluocortolone caproate and trimethylacetate; ......, fluocinolone acetonide; ——, placebo.

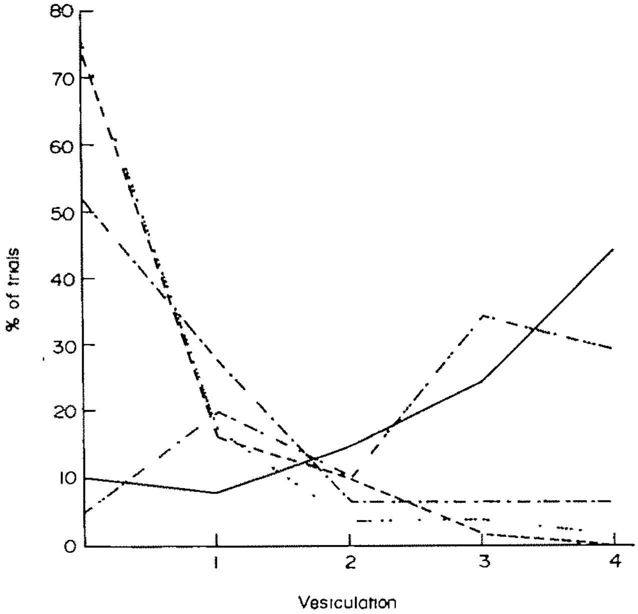

FIGURE 2. Anti-inflammatory effects of topical corticosteroids. – – – –, betamethasone 17-valerate; —·—·—·, triamcinolone acetonide; —··—··—··, fluocortolone caproate and trimethylacetate; ......, fluocinolone acetonide; ——, placebo.

RESULTS

The results of all assays are summarized in Table 1.

Vasoconstriction

All the steroids tested had significant vasoconstrictor activity and the most potent of all was betamethasone 17-valerate. There was no significant statistical difference between the triamcinolone acetonide cream, fluocortolone cream and fluocinolone acetonide cream ($P > 0.05$), but they were all significantly different from the placebo ($P < 0.01$). The placebo gave a significant number of grade I blanchings (24%) and only 2% grade II (Fig. 1).

Vesiculation

Betamethasone 17-valerate, fluocinolone acetonide and triamcinolone acetonide were equipotent in preventing vesiculation ($P > 0.05$) but they were all significantly different from the placebo ($P < 0.01$). Contrary to its relatively potent vasoconstrictor effect, fluocortolone cream was the least active of the steroids tested and was significantly different from the three other steroids ($P < 0.01$) (Fig. 2).

DISCUSSION

The simplicity of the vasoconstrictor test has led to its general use in the study of the potency of new topical corticosteroids. Although there is no direct proof that the vasoconstrictor and anti-inflammatory activities can be correlated, there is evidence that the vasoconstrictor test is of some value as an index of anti-inflammatory activity (McKenzie, 1962; Reid & Brookes, 1968). Some investigators have found difficulty in grading accurately the degree of vasoconstriction. More emphasis has been placed on the ability of corticosteroids, in high dilutions, to cause vasoconstriction, and the results have been referred to as 'present' or 'absent' vasoconstriction (McKenzie & Stoughton, 1962; McKenzie, 1966). In this double blind study, the degree of vasoconstriction, when present, was graded as 'faint' or 'definite'. There was no problem in interpreting the 'definite' blanching. Confusion, however, occurred in trying to differentiate between 'faint' and 'no' blanching reactions in certain cases, possibly because of the unavailability of a reflectance spectrophotometer (Reid & Brookes, 1968). Therefore, in evaluating the vasoconstrictor effects, the main emphasis was on the ability of the steroid to produce 'definite' blanching. No attempt was made to establish minimum effective concentrations, and all steroids used were commercial products available in different vehicles. Furthermore, this experiment differs from the classical tests in that the trials were performed on the lower back rather than on the arm. On the latter site the test is more sensitive but more difficult to interpret (Sutton, Feldman & Maibach, 1971).

When both vasoconstrictor and anti-inflammatory activities were considered, betamethasone 17-valerate was found superior to all other steroids. However, there was a great discrepancy between the vasoconstrictor effect of fluocortolone and its anti-inflammatory activity. In a study of the comparative efficacy of topical corticosteroid ointments in the blanching test, Coldman, Lockerbie & Laws (1971) found that white soft Paraffin BP gave a high placebo response at the initial reading. They attributed this to the increased hydration at the site of application. A significant part of the blanching caused by the fluocortolone cream might be due to its vehicle. Further investigations on the vehicle as well as dilution tests on the steroid itself are in order. Coldman *et al.* (1971) have suggested that hydration increases the efficacy of the corticosteroid by improving its penetration. Therefore, they concluded that the high placebo responses do not invalidate the use of the blanching test for assessing the activity of corticosteroid preparations. Our data show that there is no significant increase in the overall efficacy of the fluocortolone cream, and its anti-inflammatory effect is similar to that of the placebo. It appears from these findings that the vasoconstrictor test can be misleading if not coupled with the anti-inflammatory test or with clinical studies, especially when commercial products are being tested. This is not surprising, since knowledge of the exact mechanism involved in the vasoconstrictor effect of steroids is still meager (Thune, 1971).

In the last analysis, the important aspect is the anti-inflammatory activity rather than the vasoconstrictor potential, as the former reflects the clinical application for which the corticosteroid is ultimately destined. Therefore, considering the anti-inflammatory effects alone, betamethasone 17-valerate, fluocinolone acetonide and triamcinolone acetonide were equally active. These findings correlate well with results of previous double blind clinical studies (Zaynoun, Matta & Kurban, 1972). It is thus suggested that the Ultracin test can be of value as an index of clinical activity of topical steroids.

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Topical indomethacin and sunburn

DIANE SEKURA SNYDER AND WILLIAM H.EAGLSTEIN

Department of Dermatology, University of Miami School of Medicine, Miami, Florida 33136, U.S.A.

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SUMMARY

This study has demonstrated that a single application of a 2·5% solution of indomethacin will decrease the redness, warmth, and tenderness of sunburned skin for 24 h or longer. For this purpose indomethacin is much more effective than a highly active corticosteroid cream.

Prostaglandins appear to be important mediators of ultraviolet erythema (Greaves, Sondergaard & McDonald-Gibson, 1971; Mathur & Gandhi, 1972). We reported that prostaglandin inhibitors, indomethacin and aspirin, injected intradermally, delay and decrease ultraviolet erythema (Snyder & Eaglstein, 1973). In this study we have evaluated the effects of topically applied indomethacin and a corticosteroid on sunburn induced by a sunlamp or by natural sunlight.

MATERIALS AND METHODS

Ultraviolet irradiation

Eight normal adult volunteers were irradiated on previously untanned buttock skin. Five were exposed to sunlight, and three to a Westinghouse FS 20 sunlamp. This lamp emits ultraviolet radiation (UVR) in the 280–350 nm range; its major output is between 290 and 320 nm, the sunburn spectrum. All volunteers were sunburned by three or more MEDs (minimal erythema doses) of light. Following irradiation, the skin was not exposed to further UVR for the duration of the study.

Test agent applications

The following agents were evaluated: (1) indomethacin 2·5% w/v in the vehicle; (2) fluocinonide 0·05% cream; and (3) the vehicle alone, absolute ethanol:propylene glycol:dimethylacetamide 19:19:2 v/v. The amounts used were: 20 μl of the indomethacin solution, 15 mg of the steroid cream, and 20 μl of the vehicle. The preparations were applied to 3 cm diameter circular areas of the irradiated buttock, 1·5 h after redness was first noted. The applications were randomized to rule out regional variation.

Grading system

The red irradiated areas at sites of application of the agents were evaluated for decreased intensity of redness. Blanch intensity was graded on a 0–3+ scale: 0, no blanching; 1+, faint blanching; 2+, definite blanching but redder than non-irradiated buttock skin; and 3+, equivalent to non-irradiated buttock skin. All doubtful responses were graded as 0. Differences in temperature could be determined by touch. The skin temperature was measured with a model 43TA tele-thermometer with a flat skin probe (Yellow-Springs Instrument Co.). Differences in perception in the UVR reddened and the blanched areas were determined by stroking with the blunt point of a stylus.

RESULTS

The lightened areas (approximately 3 cm in diameter) appeared between 2·5 and 4 h after application of the agents (Plate 1). The source of UVR had no effect on the volunteers' responses.

All preparations caused at least transitory blanching in some volunteers. The vehicle alone blanched UVR redness, lasting only several hours, in three of the eight volunteers. The steroid cream produced faint blanching in three volunteers, but only one area blanched by the steroid lasted for more than 4 h. Indomethacin blanched the redness in all of the volunteers and those areas remained blanched for at least 24 h. As seen in Table 1, indomethacin was clearly the most effective agent.

The average maximum intensity of response of the blanched areas was determined for each test agent by summing the highest score attained by each subject and dividing that sum by the total number of volunteers tested. All blanched areas felt cooler than the adjacent red skin. Tele-thermometer readings showed a 0·83–1·1°C temperature difference. Most of the blanched areas felt warmer than the non-irradiated buttock skin. Tele-thermometer readings demonstrated that the blanched areas were 0·28–0·83°C warmer than non-irradiated buttock skin. The UVR reddened skin was clearly more sensitive to a stylus stroke than were the blanched areas in all volunteers.

TABLE 1. The ability of indomethacin, vehicle, and steroid to blanch sunburn

Test agents	Indomethacin solution	Solvent vehicle	Steroid cream*
No. of volunteers sunburned	8	8	8
No. of volunteers whose sunburn blanched	8	3	3
No. of volunteers with blanched sunburn at 4 h	8	3	1
No. of volunteers with blanched sunburn at 24 h	8	0	1
Average maximum intensity of response of the blanched areas†	2 2	0·5	0·5

* Fluocinonide 0·05%.

† Based on a grading scale of: 0, no blanching; 1+, faint blanching; 2+, definite blanching but redder than non-irradiated skin; and 3+, equivalent to non-irradiated skin.

Two volunteers were observed several weeks after the 24 h evaluation. The irradiated areas of their buttocks were tanned, but the sites blanched by indomethacin were less pigmented.

DISCUSSION

Although the mechanism of indomethacin's effect on sunburn is not established, its ability to inhibit prostaglandin synthetase (Ziboh, McElligott & Hsia, 1972; Greaves & McDonald-Gibson, 1973) and the possible role of prostaglandins as mediators of sunburn (Greaves *et al.*, 1971; Mathur & Gandhi, 1972) suggest indomethacin may be affecting sunburn by preventing biosynthesis of prostaglandins.

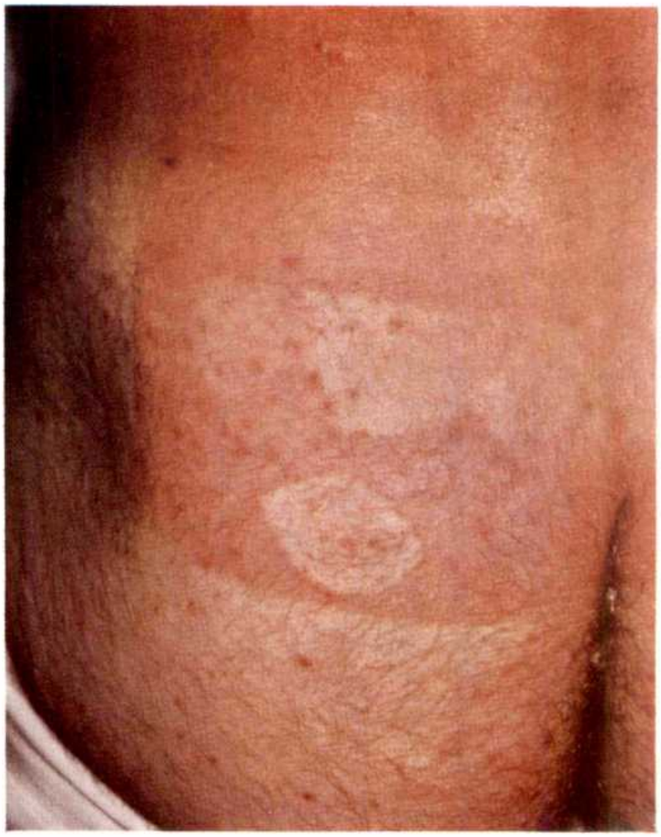

PLATE 1. Appearance of blanched sunburn 3·5 h after treatment with indomethacin solution, vehicle solvent and corticosteroid cream. The two central blanched areas were treated with indomethacin solution and are equivalent in colour to the non-irradiated buttock skin. The vehicle alone produced the faintly lightened area on the right. The steroid cream had no effect.

(Facing p. 92)

The kinetics of indomethacin's effect when applied topically are a function of its ability to penetrate the skin and of its pharmacological action within the skin.

Observations on tanning were made in two volunteers. However, the decreased tan noted at the sites blanched by indomethacin suggests that indomethacin may interfere with UVR stimulated melanogenesis. As hyperpigmentation is not seen in skin reddened by intradermal injections of prostaglandin, it seems unlikely that indomethacin interferes with melanogenesis by preventing the synthesis of prostaglandin. Topical indomethacin might prove useful in separating the direct effects of UVR on melanocytes from possible indirect effects of UVR on melanogenesis.

Before topical indomethacin can be widely used, it remains necessary to evaluate optimal concentrations, vehicles, and possible systemic toxicity. Other non-steroidal anti-inflammatory agents should also be tested. Topical indomethacin is clearly superior to one of the most potent steroid creams in decreasing the inflammatory response induced by UVR (sunburn). The therapeutic implications of this observation are obvious.

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Treatment of porphyria cutanea tarda with chloroquine

V.KORDAČ AND M.SEMRÁDOVÁ

1st Clinic of Internal Medicine (Head: Professor F.Heřmanský), and
2nd Clinic for the Diseases of the Skin (Head: Professor Z.Šťava),
Faculty of General Medicine, Charles University, Prague

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SUMMARY

Twenty men and one woman with porphyria cutanea tarda were treated with oral chloroquine, 125 mg twice weekly.

In nineteen of the patients chloroquine was taken for 8·5 months on average before complete clinical and biochemical remission occurred. Initially, treatment led to a considerable increase in urinary uroporphyrin and a mild increase in serum transaminases, but no adverse clinical reactions were observed. As the mechanism of chloroquine action in porphyria cutanea tarda has not yet been clarified, special caution must be observed when it is used therapeutically.

The best results in the treatment of porphyria cutanea tarda (PCT) have so far been achieved by the method of repeated venesections (Ippen, 1961, 1971). However, because of some disadvantages of this procedure, other therapeutic approaches have been sought.

The administration to patients with PCT of chloroquine in daily doses of 0·5–1·0 g may be followed by massive uroporphyrinuria associated with an acute reaction, persisting for several days and consisting of pyrexia, malaise, anorexia, nausea, vomiting, abdominal pain, and associated with increased levels of serum transaminases. After an interval of several months these patients had improved both clinically and biochemically. In some patients, however, the acute reaction was so severe that the authors considered it unwise to recommend this as a therapeutic procedure (Felsher & Redeker, 1966; Sweeney *et al.*, 1965).

In 1968, Saltzer, Redeker & Wilson used prolonged administration of smaller doses of chloroquine; this had some beneficial effects without adverse toxic reaction in one patient with PCT who was given 0·5 g of chloroquine twice a week over a period of more than 7 months. Similar favourable results were reported by Hunter & Donald in 1970.

Our own experience with this therapy is presented in this paper.

MATERIAL AND METHODS

Our series consisted originally of twenty-four patients with PCT; the clinical diagnosis was confirmed by biochemical examinations according to the criteria given by Gajdos & Gajdos-Török (1969).

The patients were treated with chloroquine (Delagil) administered orally in doses of 125 mg twice weekly (on Mondays and on Thursdays). Three patients defaulted and were excluded from the series. Many patients continued to take alcohol despite our advice to the contrary.

The final series thus consisted of twenty men and one woman; the average age of the men was 46·7 years (range 27–71 years), the woman was 58 years old. Our follow-up of these patients at regular intervals consisted of clinical examination, quantitative determination of urinary uro- and coproporphyrin excretion, measurement of serum iron level, transaminase levels, alkaline phosphatase, thymol turbidity reaction, Brdička reaction (polarographic determination of serum mucoproteins), cholesterol levels, and electrophoretic analysis of serum proteins. Serum transaminases were determined by the method of Reitman & Frankel (1957); upper limits of normal for SGPT and SGOT, are 30 units and 40 units, respectively. The urinary porphyrin excretion was determined according to Rimington (1958); upper limit of normal is 25 μg/24 h for uroporphyrin and 160 μg/24 h for coproporphyrin.

RESULTS

The period of chloroquine administration necessary for clinical and biochemical remission was not related to the duration of the disease, nor was there correlation between the severity of skin lesions and the quantitative values of urinary uroporphyrin excretion.

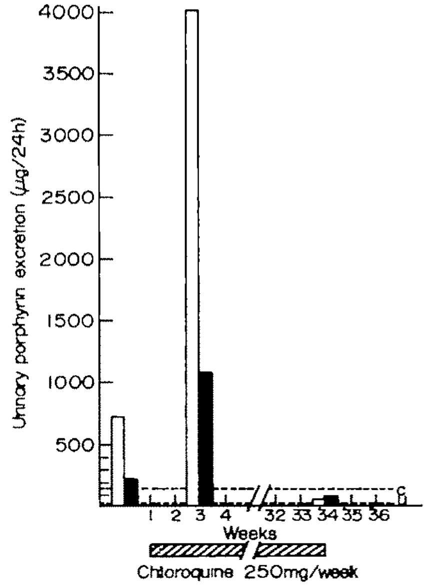

FIGURE 1. Average initial, maximum and final values of urinary uroporphyrin and coproporphyrin excretion in μg/24 h in nineteen patients during chloroquine treatment. Dashed line denotes upper limits of normal values of uroporphyrin (U) and coproporphyrin (C) excretion. Open columns, uroporphyrin; closed columns, coproporphyrin.

The duration of chloroquine administration to individual patients depended upon when improvement of clinical and biochemical abnormalities occurred. On average, chloroquine administration lasted 8·5 months, the range being 4–11 months. Treatment was discontinued as soon as the clinical and biochemical signs of the disease improved, i.e. as soon as spontaneous blisters disappeared; increased skin fragility subsided, skin hyperpigmentation receded and urinary uroporphyrin excretion fell below 75 μg/24 h.

With this therapy, clinical and biochemical remission was achieved in a total of nineteen out of twenty-one patients. All nineteen patients said that they felt better both physically and mentally. In the remaining two patients, clinical and biochemical signs of the disease failed to disappear, although chloroquine was continued in the first for 12 months, and in the second for 14 months.

TABLE 1. Urinary uroporphyrin and coproporphyrin values before, during and after treatment

Urinary excretion	Values before treatment	Maximum values during treatment	Values after cessation of treatment
Uroporphyrin (μg/24 h)			
Range	454–1506	2873–7286	31–75
Average	730	4070	70
Standard deviation	65·57	275·06	2·74
Coproporphyrin (μg/24 h)			
Range	177–308	865–1532	89–156
Average	220	1105	102
Standard deviation	8·17	41 57	4·18

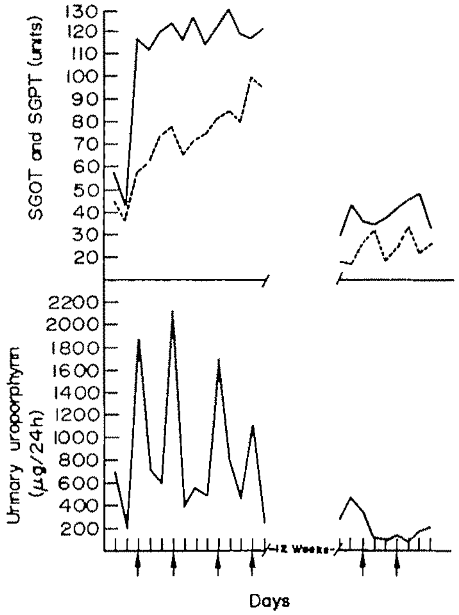

FIGURE 2. Changes in serum transaminase levels and urinary uroporphyrin found in patient V.P. (male, aged 42) at the beginning of chloroquine administration and after 12 weeks of therapy. Days when chloroquine 125 mg was administered denoted with arrows. - - - -, SGOT; ——, SGPT.

Three patients showed initial improvement with subsequent deterioration clinically and biochemically; one in the fourth month, the other two in the sixth month of treatment. Remission eventually occurred in the first two patients during the tenth and eleventh month, whilst in the third, signs of activity of the disease persisted up to the fourteenth month of treatment.

In the initial phase of the treatment there was a considerable increase in urinary uroporphyrin excretion, which usually reached a peak during the third week. Average initial, maximum and final values of urinary uroporphyrin and coproporphyrin excretion in the nineteen patients are given in Fig. 1. The range of these values is given in Table 1.

Urinary uroporphyrin excretion in individual patients varied considerably and the rate of fall to the final values was irregular.

High values of urinary uroporphyrin excretion immediately after chloroquine administration and in the initial phase of treatment were only of short duration (24–48 h). Serum transaminase levels were increased during the period of the highest urinary uroporphyrin excretion and the increase persisted for a longer time. SGPT levels were always higher than SGOT levels. After several weeks, however, the administration of chloroquine was no longer followed by immediate changes in urinary uroporphyrin excretion, and serum transaminase levels permanently remained within normal limits. These relationships are depicted in Fig. 2.

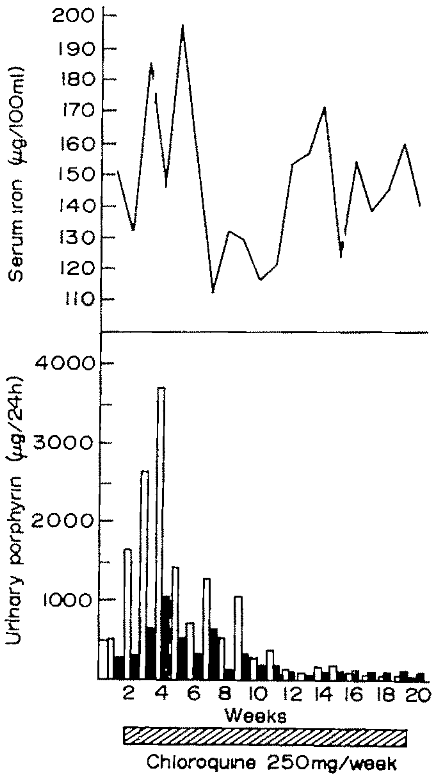

FIGURE 3. Changes in serum iron levels and in urinary uroporphyrin and coproporphyrin during the course of chloroquine treatment in patient V.H. (male, aged 49). Open columns, uroporphyrin; closed columns, coproporphyrin.

The small increase in serum iron levels observed in some patients coincided in time with the highest urinary uroporphyrin excretion (Fig. 3), but differences between the average initial and final values of serum iron levels were not statistically significant.

Serum alkaline phosphatase, thymol turbidity, Brdička reaction, cholesterol, and the electrophoretic pattern of serum proteins did not show any statistically significant variations during the treatment.

No adverse toxic reactions were observed in any of our patients during the whole period of chloroquine administration.

No eye examinations were performed; none of our patients had any symptoms referable to the eyes. Even though the doses of chloroquine are very low (1 g/month), ocular examinations will be performed in future studies.

DISCUSSION

Favourable therapeutic results with small doses of chloroquine in eight PCT patients were reported by Taljaard *et al.* in 1972, with adverse reaction in only one of their patients. These authors, however, used a dosage of chloroquine twice as high as ours. Perhaps for this reason the treatment period in our patients was much longer, but we saw no adverse reactions.

On the other hand, our low dosage of chloroquine (250 mg chloroquine weekly) might explain the poor results in two of our patients; even prolonged administration did not achieve a desirable clinical and biochemical effect. In this connection, the findings of Sweeney *et al.* (1965) seem to be important; they failed to produce any response in the urinary uroporphyrin excretion and serum transaminase levels after the second and subsequent administration of chloroquine to patients with PCT within a period of several weeks, although such a response was readily obtained after the first administration of chloroquine.

The mechanism of chloroquine action in patients with PCT has not yet been clarified. Some authors suggest the formation of a chloroquine–porphyrin complex, subsequently inducing reduction of uroporphyrin content in the liver (Felsher & Redeker, 1966); others suppose chloroquine might influence iron metabolism in the liver (Taljaard *et al.*, 1972).

Small doses of antimalarial drugs in the treatment of PCT certainly increases our therapeutic range. Treatment by repeated venesections is undoubtedly effective but it is time-consuming both for the patient and the physician, especially with large numbers of patients. If a patient is already anaemic treatment by venesection may not be advisable.

Our series of twenty-four PCT patients, who were chosen for chloroquine therapy, consisted of those treated with B-group vitamins over a period of 1–3 years without achieving any improvement in clinical and biochemical signs. Though no separate control group was included in our series we can consider each of our patients to have acted as his own control; the improvement in clinical and biochemical signs was achieved only after the chloroquine therapy. Spontaneous remission of PCT does occur, but it seems very unlikely that this was responsible for the improvement seen in our nineteen patients. We therefore suggest that with small doses of antimalarial drugs, clinical and biochemical remission can safely be achieved, especially if patients are carefully chosen.

Before a wider use of this method can be recommended, some problems remain to be solved. First, there is the choice of the most suitable dosage for long-term administration; second, the possibility that the patient might become refractory to this kind of treatment.

Finally, it must be remembered that even very small doses of chloroquine (250 mg weekly) at the beginning of the treatment induce in most patients an increase of serum transaminase levels, although the increase is mild and transient. Though the toxic effect of antimalarial drugs on liver tissue now seems to have been rather overestimated, special attention undoubtedly must be given to patients with PCT when even small doses of chloroquine are administered. No long-term results of this therapy are available at present and detailed follow-up of such patients over a period of several years will be necessary before any definite conclusions can be made. For the time being, this therapy should be performed only in specialized departments, under close supervision and with careful clinical and biochemical examinations.

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Comment

AEROBIOLOGY AND ATOPY

Comment on aerobiology and atopy is especially apposite since 1973 was the Centenary year of the publication of 'Experimental Studies on the Nature and Causes of Catarrhus Aestivus (Hay Fever or Hay Asthma)' by C. H. Blackley. Blackley's book was based on 10 years of observation and experiment upon himself, and he was the first to prove that grass pollen caused summer hay fever. In addition to this, he pioneered the use of the diagnostic skin, conjunctival, nasal and respiratory challenge tests. After an experiment in which he inhaled fungal spores, he described his symptoms so clearly that they can be classified as a Type III pulmonary reaction (in the sense of Gell & Coombs, 1968).

The term atopy (meaning a strange disease) was used by Coca (1931) to include forms of human hypersensitiveness which were subject to hereditary influence. He included asthma and hay fever and thought that eczema would eventually be placed with these. Today atopy is generally used to describe the Type I anaphylactic or reagin-dependent hypersensitivity of Gell & Coombs.

Since Blackley sampled air for pollen grains and correlated the severity of his own symptoms with the numbers of grains trapped, he may be said to have pioneered aerobiology. Aerobiology can be defined as a study of bio-aerosols. In a bio-aerosol the suspended particles are of biological origin. They are produced by plants and animals, including man, and whilst bacterial and viral aerosols cause disease by infection, other particles ranging from pollen grains to squamous epithelium cause disease because they are antigenic. Such particles, although antigenic to the normal subject, produce disease only in persons who have developed reaginic or Class IgE antibodies. Consideration is, therefore, given to the aero-allergens which may play a role in the aetiology of atopic skin disease.

Outdoors, bio-aerosols have their origins in or on the surfaces of vegetation, and as the vegetative cover changes with the seasons of the year, there are great changes in the content of the air. The diagnosis of seasonal atopic disease must, therefore, depend in the first instance upon a knowledge of when the patient is likely to be exposed to various airborne allergens, and one of the major developments since Blackley's studies was the invention of the Cascade Impactor by May (1945). 'Fall-out' or 'sedimentary sampling' in which a sticky-faced slide is exposed horizontally is inadequate (Davies, 1971). The Cascade Impactor is a suction sampler which fractionates the trapped particles according to size in a manner similar to the respiratory tract. Particles larger than spheres of 7 μm diameter and unit density are deposited in the upper respiratory tract; 50% of those 3·8 μm in diameter reach the alveoli of the lung (Druett, 1967). The automatic volumetric trap of Hirst (1952), which is based on the second stage of the Cascade Impactor, has a 100% retentiveness for particles of 7·1 μm diameter and simulates the upper respiratory tract with respect to the deposition of pollens and other particles which produce rhinitis and asthma in the allergic subject. It must be emphasized that grass pollen grains, which are rarely smaller than 25 μm, are not inhaled into any portion of the lower respiratory tract (Wilson *et al.*, 1973) where the airway obstruction occurs. Moreover, plasma cells which fluoresce with anti-IgE are sparse in the lung, but occur in the bronchial and tracheal mucosa, and are present in greatest numbers in the tonsils and adenoids (Ishizaka & Ishizaka, 1970). They considered that the presence of many IgE-forming plasma cells in adenoids and tonsils taken at surgical operation, suggests proliferation due to local antigenic stimulation. They also reported the occurrence of IgE-forming plasma cells in the gastrointestinal tract, and it should be noted that in addition to foods, inhalent allergens are also ingested.

The Hirst Trap is at present the apparatus of choice for surveys into the fungal spore and the pollen content of the ambient atmosphere, because it is a continuous sampler (Davies, 1971). Among a number of studies in which this apparatus was used are those by Hirst (1953), Hyde (1959) and Davies (1969a, 1969b). 'The Microbiology of the Atmosphere', by P. H. Gregory (1973), is a valuable text which includes chapters on: 'The air spora near the earth's surface', 'The air spora of enclosed spaces', and 'Inhaled microbes'.

In Britain in the Spring, the catkin-bearing trees disseminate pollens, of which the most important is birch (*Betula*). In the Summer, especially between June and July, clouds of grass pollens develop, and although nettle (*Urtica*) at this time is numerically second in importance to the grasses, it is grass pollen which causes most trouble. The amount of grass pollen in the air is weather dependent. Rain is important, especially if it falls in the afternoon when it 'washes the air clean', and temperature for growth in April and May determines the onset of the June/July season. A method of forecasting the start and severity of the hay fever season using April and May temperatures is described by Davies & Smith (1973).

Between early summer and late autumn, varying diurnally with the weather, clouds of fungal spores become airborne in far higher concentrations than those recorded for pollens.

Feinberg (1944) found that in Chicago, 20% of his allergic patients were allergic to some kind of mould or other. Hyde, Richards & Williams (1956), in an important study on moulds and summer asthma, reported that in seasonal asthma due to moulds, there is little or no rhinitis or conjunctivitis, as is the case with pollen. One explanation for this may be that whereas the mass of the pollen grain is large enough for it to be impacted on to the conjunctiva with low air velocities, that of fungal spores is too small. Hyde *et al.* found that 4% of 627 patients were only allergic to the ubiquitous mould *Cladosporium*. They concluded that many cases of summer asthma were due to fungal spores, especially *Cladosporium*; and 'that allergy to mould spores in all probability accounts for some 5% of all cases of asthma and may well play a subsidiary part in many more'. During warm weather, in the air over London, *Cladosporium* concentrations reach 10^4 spores/m^3 and *Alternaria* 10^2 or rarely $10^3/m^3$. Cereal crops are prolific sources of fungal spores, and although a number require further investigation as allergens, it may be said that the genera *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* should always be considered as a cause of mould allergy.

Although in the high concentrations attained, fungal spores represent the predominant microbe in the air outdoors, the spores of mosses, ferns, the cells of terrestrial algae and the propagules of lichens also occur. Hay fever symptoms due to algae were described by Heise (1949), and McGovern, McElhanney & Brown (1965), in a study on the allergenicity of algae, reported that high aerial concentrations occurred during dust storms in Texas. Champion (1971) has observed atopic disease due to algae and lichens in East Anglia, an area where soil erosion has been reported. In the air over London, algal concentrations are highest on windy days, and very rarely attain $10^2/m^3$.

Indoors, with natural ventilation to the ambient atmosphere, the seasonally occurring pollens and spores from the air outside are present, but in very much lower concentrations (Hyde *et al.*, 1956; Davies, 1969b). In addition to this, man by his activities contaminates the intramural environment. His desquamated skin scales are common in the air and comprise about 20% of the total weight of house dust samples collected in routine vacuum cleaning (Davies, 1958). Kern (1921), who originally described house dust sensitization, considered it to be the commonest single cause of asthma in adults. Voorhorst, Spieksma & Varekamp (1969) reported the mite *Dermatophagoides pteronyssinus* to be the most important allergen in house dust, and that the skin content of the dust is its nutrient source. These workers reported a rise in the mite content of the dust in September–November, and correlated this with a seasonal increase in asthma in the Netherlands. The mite content of dust from many countries is reported by Frankland (1972), who found no mites in the relatively dust-free wards of three London hospitals. It is noteworthy that the mite is not normally airborne, and it is possible that allergic sensitivity is to the faeces, as is the case in locust sensitivity described by Frankland (1953). Kern described house dust asthma as perennial, and in the U.S.A., with effective air conditioning, the intramural climate is remarkably constant. However, air conditioning systems may themselves be a source of antigens as ducts may become colonized by moulds, and Banasak, Thiede & Fink (1970) described hypersensitivity pneumonitis due to the contamination of an air conditioner with the thermophilic actinomycete which causes Farmer's Lung (Pepys *et al.*, 1963).

The damp house may present special problems. When timber is infected with dry-rot, the spores of *Merulius lacrymans* can occur in high concentrations and cause allergic disease (Frankland & Hay, 1951). Moulds are the 'weeds of the home' (Brown, 1936); algae proliferate in moist situations and Bernstein & Safferman (1970) considered the algal content of house dust to be important in dust sensitivity.

During the latter half of the century since Blackley's publication, aerobiological knowledge has greatly expanded, and the importance of climate and environment in atopic dermatitis has been noted by such authorities as Baagoe (1949) and Champion & Parish (1972). Champion (1971) has observed seasonal exacerbations of atopic dermatitis in grass pollen sensitive patients, and although such reports appear to be rare, the asthma–eczema syndrome is well recognized. Surveys of the prevalence of eczema and asthma in children are described by Walker & Warin (1956) and Brereton *et al.* (1959).

Experimental studies which show skin disease being provoked by bronchial or nasal challenge were reported by Sulzberger & Vaughn (1934) and Tuft, Tuft & Heck (1950). Sulzberger & Vaughn described a woman sensitive to silk, and they injected some of her serum into the skin of a normal subject in the manner of a PK test. After 48 h the test subject sniffed a little powdered silk into each nostril, and about 18 min later experienced intense itching in the atopic serum injected sites. A small weal and areola of erythema had developed after 20 min, and 35 min after sniffing the silk the weals were about 2 inches in diameter. After 40 min the itching began to subside, and nothing happened at the sites where normal serum had been injected. Tuft *et al.* observed that in a patient sensitive to *Alternaria*, challenge by inhalation with a dry powdered preparation of the mould produced an immediate nasal reaction, and a systemic reaction which consisted of profuse sweating in areas where skin lesions had been present. An outbreak of dermatitis occurred within 24 h of the test and lasted 4–5 days. They found the outbreaks of dermatitis could be provoked when he was symptom free, but were not provoked when he was exposed to pollens and particles to which he was not allergic.

Although the removal of the patient to an allergen-free environment produces a relief of atopic dermatitis (Nexmand, cited by Baagoe, 1949), and this may be considered to be evidence that aero-allergens cause dermatitis, this treatment is usually accompanied by the relief of the patient's asthma. However, it may be concluded that allergens act on the atopic subject as a whole. Reference has already been made to the inhalation and deposition of particles in the upper respiratory tract, and that they produce obstruction of airways in the non-ciliated alveolar regions of the lung. Evidence of an airborne allergen producing another systemic effect is given in the nephrotic syndrome of Hardwicke *et al.* (1959).

Since the inhaled pollen or other particle is deposited upon a ciliated mucous membrane, and it is through this that the IgE reacting antigen is absorbed, Taylor *et al.* (1973) have examined the protective effect of IgA, the principal immunoglobulin of secretions. They reported that IgA deficiency at 3 months was associated with the development of atopy (eczema and positive prick tests) within the first year in the offspring of atopic parents. They suggested that transient IgA deficiency in infancy could lead to atopic disease if a normally responsive IgE system were overstimulated by antigen during an IgA deficient period. These findings suggest that a brief period of allergen avoidance during a symptom-free vulnerable period might prevent much atopic disease. Aerobiology provides a basis of knowledge for allergen avoidance, and the effect of an allergen-reduced environment in the prevention of the development and in treatment of atopic dermatitis would seem worthy of investigation.

R. R. DAVIES

Wright-Fleming Institute,
St Mary's Hospital Medical School,
Norfolk Place,
London W2 1PG

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Society Proceedings

West of England and Wales Society of Dermatology, Bristol

10 February 1973

Chairman: Dr R. P. Warin

Necrobiosis Lipoidica Diabeticorum Disseminata Dr R. P. Warin

A girl aged 18.

History. She has been a known diabetic for 4 years and had been on insulin but is now on diet only. Serum jaundice 10 weeks ago: Australia antigen isolated. Lesions started to develop 1 year ago but no new ones have appeared for 3 months.

Family history. Both her mother and maternal grandmother have mild diabetes.

On examination there are some 30–40 typical necrobiosis lesions scattered over the chest and upper part of the back; typical lesions of granuloma annulare are present on the left middle finger and on the right foot; one lesion typical of necrobiosis and one of granuloma annulare, are seen, each at sites of friction against the shoes.

Comment. Multiple lesions of necrobiosis lipoidica diabeticorum on the legs are common, but disseminated lesions on the trunk must be very rare. The lesions are quite typical, but have not been confirmed histologically because of the presence of Australia antigen in the blood. The association of necrobiosis lipoidica diabeticorum and granuloma annulare is well recognized and the latter appears to occur at sites of repeated trauma.

Pyoderma Gangrenosum of Scalp Dr R. D. G. Peachey

A woman aged 21.

History. In 1961 she developed ulcerative colitis at the age of 10 years and has had three severe episodes in the last 11 years. In 1971, coinciding with an exacerbation of her colitis, she developed pyoderma gangrenosum with ulceration on the right ankle and below the left knee. This was treated with systemic corticosteroids; the ulceration at the ankle was grafted. All lesions healed well. In July 1972, when her bowel symptoms were quiescent and controlled with sulphasalazine, she suddenly developed many painful lesions on the scalp, with crusting and discharge of pus (Fig. 1). Treatment with erythromycin and cephalexin failed to improve her condition, and she was admitted to hospital with fever, tender curvical glands, and multiple necrotic 'abscesses' on the scalp, which discharged green-yellow pus.

Family history. Sister had been diagnosed as having ulcerative colitis in 1972.

On examination there were multiple 'abscesses' and crusted, granulomatous areas scattered over a wide area of the scalp.

Investigations. Bacteriology of scalp lesion; original culture—*Pseudomonas pyocyanea* and *Staphylococcus albus*; subsequent cultures—*Ps. pyocyanea* (frequently), *Staph. aureus* (once only) and anaerobic streptococcus. Mycology—negative. Hb 12·5 g/100 ml, WBC 8400/mm^3, ESR 5 mm/1 h. Serum proteins—normal. Immunoglobulins—normal. Barium enema (August 1972): appearances of ulcerative colitis involving whole of large bowel, with exception of rectum and sigmoid colon.

Biopsy of scalp. Sections show several small superficial intra-epidermal abscesses. Elsewhere the epidermis shows slight acanthosis, and there is slight flattening and loss of rete pegs, with some separation from the dermis. There are several areas of diffuse chronic inflammatory exudate rich in plasma cells in the middle and lower dermis, with considerable fibrosis.

Treatment. Flucloxacillin was administered at the time of her admission to hospital before bacterial cultures and sensitivity reports were available. In spite of the fever, tender cervical glands, and a raised white cell count at this time (15,600 cells/mm^3, with 81% neutrophils); the only organisms isolated were *Staph. albus* and *Pyocyaneus aeruginosa.* Flucloxacillin had no apparent effect on the skin lesions but the fever and tenderness of the glands settled slowly over a period of about 10 days; this appeared to coincide with the use topically of neomycin cream. Over the last 6 months her scalp condition has run a variable course with partial healing, crusting, indolent abscess formation and areas of granulation tissue and ulceration. A 7-day course of gentamicin and carbenicillin, to which the *P. aeruginosa* was sensitive, had no beneficial effect and no improvement occurred following a course of prednisone (45 mg/day). Slow but steady improvement followed the use of 0·1% betamethasone 17-valerate lotion applied to the scalp under polythene occlusion at night.

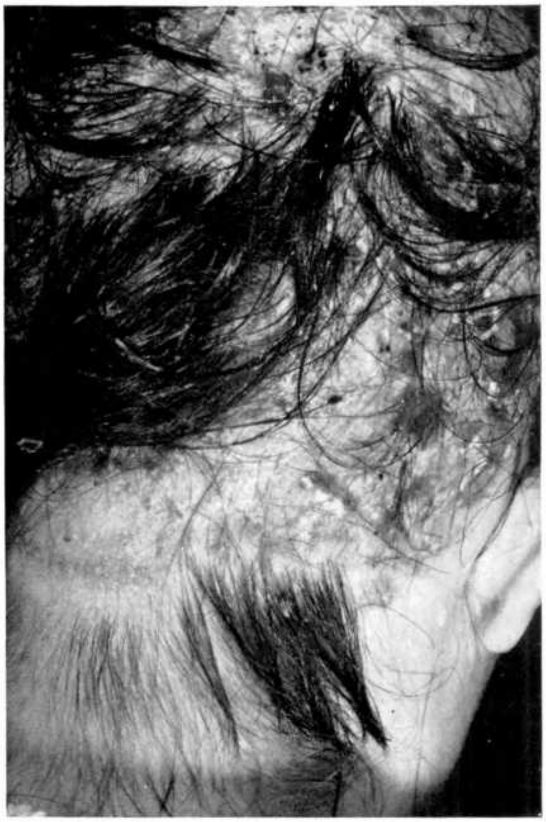

FIGURE 1. Pyoderma gangrenosum of scalp.

Comment. In spite of the unusual site, there seems little doubt that this patient had pyoderma gangrenosum involving the scalp. Associated secondary infection may have accounted, in part, for her original fever, leukocytosis, and tender cervical glands, although no proof of this was obtained. Continued treatment with 0·1% betamethasone 17-valerate lotion, originally with polythene occulsion but more recently without occlusion, has resulted in complete healing of the scalp lesions. Her ulcerative colitis has been quiescent throughout.

Implantation of Colonic Mucosa in Skin Around Colostomy DR R. D. G. PEACHEY

A boy aged 10.

History. Hirschsprung's disease was diagnosed at the age of 2 months, and a colostomy was performed at the age of $3\frac{1}{2}$ months following perforation of the colon. Further surgery was carried out between 1963 and 1969 because of recurrent faecal fistulae and adhesions. In 1971 he was referred because persistent 'granulation tissue' around the colostomy opening would not epithelialize.

On examination the colostomy was surrounded by a rim of bright red 'granulation tissue', which extended in tongue-like processes into the surrounding skin (Fig. 1). This was originally thought to be due to irritation and excoriation of the skin surrounding the colostomy, but the tongue-like areas did not alter at all over a period of time and did not improve with topical corticosteroid applications.

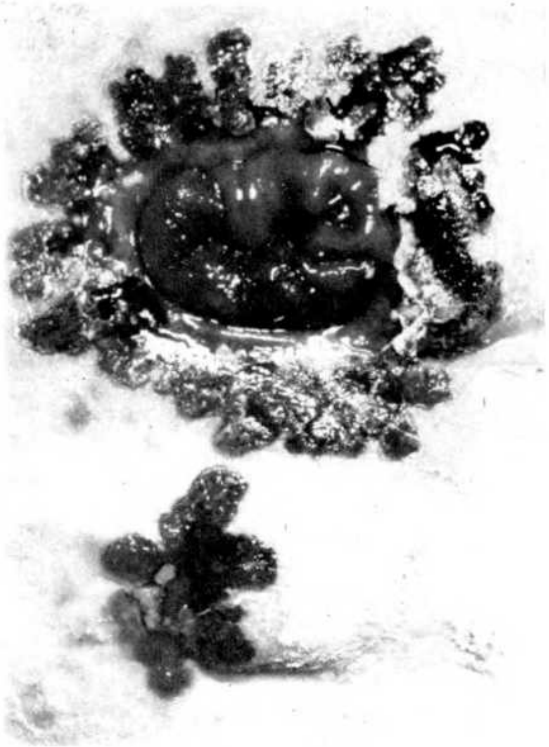

FIGURE 1.

Investigation. A biopsy was taken across one of the tongue-like areas. Chronically inflamed and ulcerated colonic mucosa could be seen to be contiguous with skin.

Comment. Implantation of colonic mucosa into the skin around the colostomy opening has occurred in this case. Cauterization of part of the implanted area with silver nitrate did not result in improvement.

Correspondence

DILUTION OF TOPICAL CORTICOSTEROID FORMULATIONS

Sir, The dilution of commercially available corticosteroid formulations is common practice in the U.K., although unknown in some other countries. We believe that *ad hoc* dilutions are, in principle, undesirable and that, if their use is considered to be essential, the choice of diluent should be based on rational considerations. Many of the 'compound' mixtures of corticosteroids with coal tar, cade oil and the like may lack both pharmaceutical stability and therapeutic justification.

The clinical efficacy of dilutions of commercial steroid formulations depends entirely on the degree of dilution, and a preparation such as Synandone, containing half the fluocinolone acetonide content of Synalar, is superior to 1% hydrocortisone. On the other hand, it is extremely unlikely that a dilution containing as little as 10% of the original formulation offers any advantage over 1% hydrocortisone.

The powerful topical anti-inflammatory steroids, such as fluocinolone acetonide or fluocinonide, are complex molecules and are present in very small amounts (Synalar ointment contains 250 p.p.m. of fluocinolone acetonide). The risk of total chemical decomposition is greater than with high strength active agents. Pharmaceutical companies spend much research effort in selecting optimum formulations, taking into consideration chemical and physical stability, together with clinical efficacy. Any alteration or addition to such a formulation can radically alter any or all of these factors. For instance, in alkaline vehicles such as Boots E45 Cream, Synalar (fluocinolone acetonide) and Metosyn (fluocinonide) undergo oxidative degradation at the 11 and 21 positions. Thus, a dilution of 20% Metosyn in E45 cream loses 50% of its steroid concentration after 1 month's storage at 23°C. The selection of an effective preservative for a topical cream is of crucial importance. Many of the preservatives commonly used in pharmaceutical emulsions are adsorbed or bound by non-ionic emulsifiers (these are usually used in corticosteroid creams) and this bound preservative is devoid of antimicrobial activity. An additional factor is the oil/water solubility (partition coefficient) of the preservative. The degree of preservation depends on the availability of the preservative in the aqueous phase. If, on dilution of a well preserved cream, further oils are added, the preservative may tend to partition into that oil phase leaving the product in a badly preserved state.

It is because of these difficulties with stability and activity that some commercial steroid preparations are issued at high and low strengths in order to overcome the necessity for extemporaneous dilution.

All the Synalar products and Metosyn are processed under sterile conditions, and any subsequent dilution, unless done aseptically with sterile ingredients, will inevitably introduce contaminating micro-organisms. This is another reason for discouraging the practice of dilution.

Ointments present a lesser problem since, being anhydrous preparations, they do not readily support the growth of micro-organisms. Nevertheless, they can be contaminated by the addition of a non-sterile product.

Dilution will, of course, continue despite these comments and the following suggestions are made to protect the interests of both the prescriber and the patient: (1) use simple, adequately preserved diluents,* (2) make small batches, (3) do not keep longer than necessary for patient treatment, (4) use aseptic techniques for mixing.

Cetomacrogol Cream, Formula B, is recommended for diluting Synalar creams and Metosyn. Ointments may be diluted with sterile white or yellow soft paraffin. To enable the preparation of pharmaceutically elegant dilutions of Metosyn, the synthetic base alone is now available in pots of 500 g.

*Information on suitable diluents for all the commonly used topical corticosteroids can be found in the *British Pharmaceutical Codex*.

A.F.Mooney

Medical Information Group, Imperial Chemical Industries Ltd,
Pharmaceuticals Division, Mereside,
Alderley Park, Macclesfield

J.D.PEARCE

Pharmaceutical Department, Imperial Chemical Industries Ltd,
Pharmaceuticals Division, Macclesfield Works,
Hurdsfield Estate, Macclesfield

HERPES ZOSTER FOLLOWING PRIMARY SMALLPOX VACCINATION

SIR, A healthy girl, aged 8, received primary smallpox vaccination on the left upper arm on 11 April 1973. Almost 2 weeks later, on 24 April, following pain over the right side of her face and neck, vesicles of herpes zoster appeared over the right second and third cervical dermatomes (Fig. 1). By this time, the successful smallpox vaccination site showed a scab. She was admitted to hospital and recovered uneventfully.

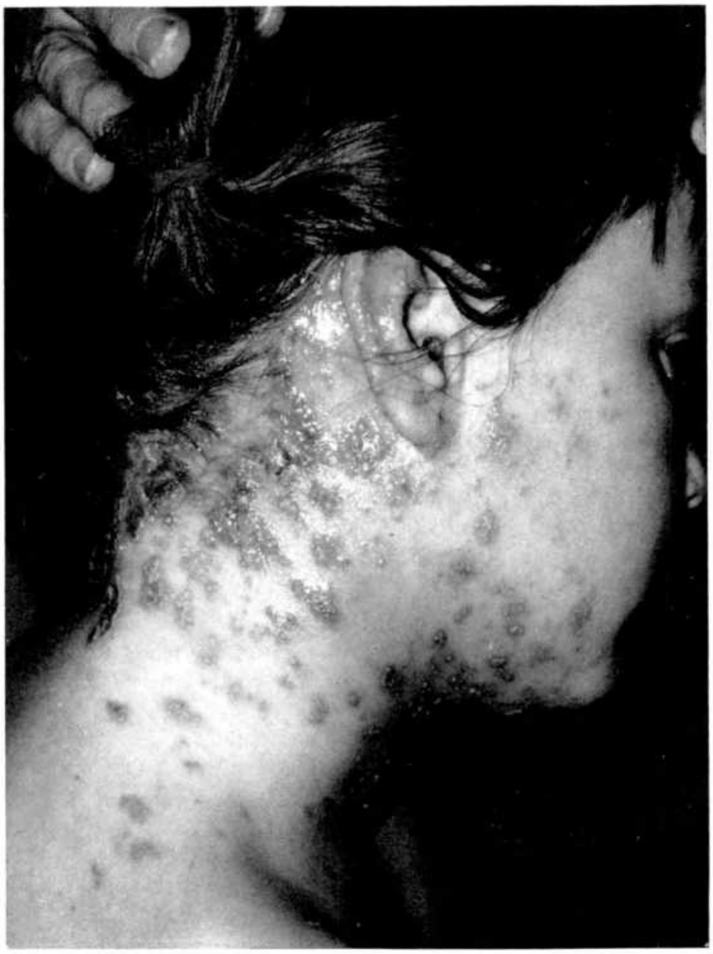

FIGURE 1.

The child had been vaccinated before a holiday abroad. She had never been vaccinated previously because of a strong family history of atopy; the patient herself had been 'wheezy' as an infant but had experienced no other atopic manifestations. Her mother thought that the child had had chickenpox when much younger, but could not remember when. There was no history of recent contact with zoster or varicella.

It seems most likely that the appearance of zoster in this patient following primary vaccination was coincidental. I could find no previous report in the literature, and Dr A. B. Christie of the Infectious Diseases Unit, Fazakerley Hospital, Liverpool, in his wide experience, has not heard of a similar case. Perhaps some systemic disturbance following the vaccination served to activate the dormant varicella-zoster virus in this child, but if this is so, one would have expected the appearance of zoster after primary vaccination to have been more common.

JULIAN VERBOV

Department of Dermatology,
Newsham General Hospital,
Belmont Road, Liverpool L6 4AF

THE HALO NAEVUS AND CUTANEOUS MALIGNANT MELANOMA

SIR, The finding by Copeman *et al.* (1973) of a common antibody in cases of malignant melanoma and halo naevus is both important and provocative, but several of the conclusions they draw about the interpretation of their findings seem open to question. For example, they postulate that because the antibody concerned is found in both the above conditions, a halo naevus is therefore a naevus developing malignant change. This appears not only to be a non-sequitur, and one which is very difficult to square up with the simple observed facts of the natural history and pathology of halo naevus, but it may also confuse further work with this antibody.

Far from behaving like a malignant or premalignant lesion, a halo naevus seems to represent the very opposite; an outstandingly clear example of involution, occurring either idiopathically or following a bout of intense sunbathing or in association with vitiligo.

The antibody concerned was first detected in cases of malignant melanoma, and hence it has been labelled 'malignant melanoma cytoplasmic humoral antibody' (MMCHA). Thence it seems to have been presumed that the antibody is specifically associated with the *malignancy* of the melanoma cells. This does not necessarily follow. There may surely be other antigenic peculiarities of malignant melanoma cells besides their indefinable quality of 'malignancy'.

Supposing history had been different and the antibody had first been discovered in halo naevi, it would then presumably have been labelled 'halo naevus cell cytoplasmic humoral antibody'. And if the same reasoning had been followed, the conclusion drawn would have been that all malignant melanomas later found to be associated with the antibody were halo naevi undergoing involution. While we are all aware that now and again a primary melanoma does undergo involution, as a generalization this conclusion would clearly be absurd.

There is a variety of obstacles to accepting halo naevi as being 'naevi undergoing malignant change'. One is their occasional multiplicity. There are patients who develop dozens of halo naevi simultaneously or within a period of a few months, and the mind-boggling novelty of such an exanthem of separate primary neoplasms is relieved only by the merciful coincidence that when such 'malignant melanomas' develop in swarms they are all doomed to failure.

Secondly, it has to be remembered that pathologists have for a long time been looking at naevi which seem to be undoubtedly becoming malignant. Apart from the presence of lymphocytes in both types of lesion there is little else in common between the histology of an early malignant melanoma and that of a halo naevus. One has the features of a proliferative process, and the other those of an involutionary one. In a naevus undergoing early malignant change there are signs of 'unrest' amongst the melanocytes in the epidermis, indications of increased cell turnover, 'junction activity' and often associated epidermal disturbance. All these, and others, may be termed 'signs of progression towards full malignancy' in a naevus. The associated lymphocyte response which is often to be seen in such a lesion, together with the accumulation of melanin (derived, one assumes, from dead or damaged melanocytes) within dermal melanophages, may be interpreted as concurrent 'signs of involution'. An early melanoma is commonly both growing and involuting at the same time. If halo naevi were, as is suggested, naevi becoming malignant, it might be expected that they would show some of the 'signs of progression', whereas in fact they seem to show only those of 'involution'.

Thirdly, it is generally believed that when a compound naevus becomes malignant it is not the intradermal naevus cells which transmute into malignant melanoma cells, but that a new race of malignant melanocytes is generated from the epidermis overlying the naevus. In an early melanoma arising from a pre-existing benign naevus there are two distinct populations of cells present, old intradermal naevus cells which were generated in childhood and new malignant cells currently proliferating from the epidermis. Yet in a halo naevus the intradermal naevus cells are destroyed; cells which are believed to be not only benign but in a sense impotent. And it is during the phase of their destruction that the antibody is present in the blood.

In brief, it would seem that halo naevi and early melanomas share at least three features; a lymphocyte reaction, the presence of a common antibody in the blood and a process involving melanocyte destruction. These three things may well be linked, but the linkage need not be dependent upon a 'malignant' process: only upon a process involving melanocyte destruction within the dermis. And while such a process could occur as a side effect of early malignancy, it could also arise in a purely involutionary context.

The danger of loose thinking which equates these two processes because of a common antibody is this. If it is assumed that the MMCHA is specifically an antibody against some 'malignant' fraction of the melanoma cell, it clearly has great implications for the future of melanoma therapy and for the whole of tumour pathology. But is there sound evidence that this antibody is to the 'malignant' quality of the tumour cells rather than to some other associated peculiarity, for example a disposition to undergo death and destruction? The discovery of the antibody in patients with halo naevi would indeed seem to cast doubt on the former and to support the latter alternative.

The malignant melanoma cell is unusual among melanocytes in that it lives and multiplies and dies within the dermis, whereas normal melanocytes carry on their turnover above the epidermal basement membrane. In ordinary compound naevi the intradermal melanocytes live long, multiply little and die slowly. The one other condition, apart from malignant melanoma, in which melanocytes die and break down in the dermis in large numbers within a short span of time is the halo naevus.

It might be simpler to suppose that the MMCHA is produced in response to large numbers of melanocytes dying and breaking down in the dermis, regardless of whether they are malignant or not. If this were so it might call not only for an alteration to the name given to the antibody, but also for a reassessment of its behavioural role in malignant melanoma.

While not attempting to put forward any firm alternative hypothesis (at present the amount of factual data seems inadequate), I am simply questioning whether the authors are correct in concluding that because the same antibody is found in malignant melanoma and halo naevus, that therefore halo naevus is a 'malignant' lesion. To do so seems dangerously like turning pathology upside down for the sake of explaining two newly observed facts. The observations concerned might perhaps equally well be explained in other ways, without so upsetting the apple-cart. For instance, it could be supposed that the common factor between the two conditions is not 'malignancy' but 'involution'; not the neoplastic proliferation of pigment cells but their instability and their proneness to death and destruction. Such an explanation would be in keeping with the authors' other observations, that the antibody was absent from children with actively proliferating naevi, from patients with benign juvenile melanomas and from patients whose naevi were inflamed but not involuting. Furthermore, it might help to account for why in a halo naevus there is (in my experience) no lymphocyte reaction in the region of the halo itself, where depigmentation occurs, but where the melanocytes concerned all lie above the basement membrane. The lymphocytes are quite sharply confined to that part of the dermis which contains naevus cells.

There are known circumstances in which a primarily 'non-immunological' event, such as traumatic tissue damage, can initiate a progressively destructive immunological process, e.g. in the eye (sympathetic ophthalmia), in the testis and in the central nervous system. It is perhaps possible that the immunological link between halo naevus and malignant melanoma may be of this nature.

I.W.WHIMSTER

Department of Dermatology,
St Thomas' Hospital,
London SE1 7EH

REFERENCE

COPEMAN, P.W.M., LEWIS, M.G., PHILLIPS, T.M. & ELLIOTT, P.G. (1973) Immunological association of the halo naevus with cutaneous malignant melanoma. *British Journal of Dermatology*, 88, 127.

Book Reviews

Neonatal Dermatology. L.M.SOLOMON AND N.B.ESTERLEY. Volume IX in the series—**Major Problems in Clinical Pediatrics.** ALEXANDER J.SCHAFFER (Ed.) (1973) Philadelphia, London, Toronto: Saunders. Pp. xi+214, illustrated. Price £7.05.

This is an excellent book, well illustrated in black and white and colour, with many references to literature published predominantly between the years 1960 and 1971. It is well balanced, including many rarities, but also sensible statements on the management of minor and common neonatal skin diseases. Most of the text concerns skin problems which require attention well beyond the neonatal period. Since it is part of the series 'Major Problems in Clinical Pediatrics', it will probably find a place on the paediatric bookshelves and, bearing this in mind, there is little to criticise from the dermatologist's point of view. Perhaps there should be a chapter more specifically concerned with genetic counselling; more might be written also about purpura fulminans, disseminated intravascular coagulation, leukaemia, lipoid proteinosis and balanitis. The debate about ammoniacal dermatitis and napkin psoriasis deserves mention. I disagree with the emphasis on punch rather than excision biopsy. I do not think excision of juvenile melanoma is necessary.

Nor do I think that it is true to say that there are five plexuses of veins in the new born skin. Some parts of the book, such as that describing the appearance of the new born skin, the explanation of linear lesions as due to cutaneous vessels or nerves, and that on the management of juvenile dermatitis herpetiformis, seem inadequate. Some of the black and white figures are useless, *vide* 3-3, 3-10, and 8-6. The reference in the text to Mulvihill on p. 14 is missing in the list of references, and it should be Beare, not Beane, on p. 62. However, it will be seen that on careful reading, one can find few serious blemishes. With a struggle one could think of a few extra rarities but these would unbalance a useful book.

T.J.RYAN

Handbuch der Speziellen pathologischen Anatomie und Histologie. Vol. 7. **Weibliche Geschlechtsorgane.** E.UEHLINGER (Ed.) Part 4. **Vulva, Vagina, Urethra.** G.DALLENBACH-HELLWEG, B.EGLOFF, H.G.HILLEMANNS, J.JAEGER, G.F.KLOSTERMANN, H.LIMBURG, K.STAFFELDT AND P.STOLL (1972) Berlin, Heidelberg, New York: Springer. Pp. xvii+914, 378 illustrations. Price £53.73.

This massive work is the final volume in a series on special pathology begun in 1924. It deals with the cytology of the female genital tract; dermatoses of the vulva, including venereal diseases, and the involvement of the vagina in these conditions; the pathology of the urethra; tumours and cysts of the vulva, and its other disorders—such as atrophy, oedema, varices and injuries; tumours and cysts of the vagina; and finally dysplasia and carcinoma-in-situ of the cervix. As is to be expected, the emphasis is on pathology, but clinical aspects are not ignored.

The problems of multiple authorship show not only in the overlapping of subject matter from section to section without cross-references, and in the inconsistent conventions of typography, but in marked differences as regards consideration of recent material.

All the sections are solidly grounded in history and accepted fact. Those on the vagina and cervix are in addition admirably up-to-date; thus in considering the aetiological factors in cervical carcinoma, the possible significance of herpesvirus infections is discussed, and similarly the suspicion that stilboestrol given to the mother may cause adenocarcinoma of the vagina in the adolescent girl is noted. The sections of more direct concern to the dermatologist, however, are less complete. It is disappointing, for example, to find a discussion on Paget's disease which omits much recent work on the nature and origin of the Paget cell, and to see that erythrasma is still classified as a fungal disease. Pemphigus is discussed without reference to work on immunopathology. Warts are included in several sections and a more co-ordinated approach would be an improvement. Their malignant potential is noted but not described in any detail. It is of interest to note that the 'true papilloma', i.e. a non-viral papilloma, is described as a distinct entity. Lichen sclerosus is a touchstone by which dermatologists may judge a work. Here it is discussed briefly by a dermatologist, and at length in relation to 'kraurosis' and leukoplakia by a pathologist. Again, a single account with a cross reference would have been more satisfactory; and it is a pity that little attention is paid to the proposals made in British publications for clarification of the issue.

The photographs, whether clinical or histological, are superb, and in them lies much of the value of the book. The lists of references at the end of each section—sometimes but not often classified under separate general headings—are almost dauntingly long, occupying several pages each (twenty-five in the case of the section on dermatoses of the vulva). However, with the exceptions mentioned, they tend not to cover the last 10 years or more. There is a useful author index, but the subject index is occasionally inadequate or even inaccurate—a serious criticism in a book which is par excellence one for reference.

Nevertheless, as a source of reference it will be invaluable, particularly to those seeking out details of rare conditions, whether it be primarily for the histological findings or for a comprehensive account of previous reports.

C.M.RIDLEY

Rheumatology, An Annual Review, Vol. 4, **Oculocutaneous Manifestations of Rheumatic Diseases.** G. E. EHRLICH (Volume Ed.) (1973) Basel: S. Karger. Price £8.85.

This volume is divided into three distinct sections compiled by different groups of authors. The first deals with ocular manifestations of rheumatic disorders, and also with iatrogenic ocular problems encountered in the course of treatment of rheumatological disorders; the second performs the same function with regard to oral–mucosal problems in rheumatology. The authors of the dermatological section appear, however, to have been given a different brief, as this section is devoted to the skin as a reflector of immunological change. It deals, necessarily briefly, with immunological abnormalities observed in a wide spectrum of dermatological disorders, including such diseases as vitiligo and pemphigus, in addition to the skin lesions commonly associated with rheumatological problems. A comprehensive introduction by G.E. Ehrlich stresses the fascinating overlap of diseases within the spectrum of collagen or connective tissue problems, and is, like the three main sections, well provided with an excellent selection of current references.

Ocular problems are comprehensively dealt with. The discussion on changes observed in the eye in systemic lupus erythematosus is of particular interest in view of the varying incidence of ocular involvement quoted in this disorder. It stresses the point in this section which emerges as of prime importance to the dermatologist, that in any multisystem disorder, the opinion of an ophthalmologist should be sought at the time of presentation rather than when therapy may have radically altered ocular signs and symptoms. The section on iatrogenic ocular problems includes a reminder on the need for regular supervision of all patients receiving systemic corticosteroids and antimalarials.

Oral-mucosal disorders in relation to rheumatology are discussed in a similar clear and logical fashion. These entities are divided into four groups: those in which oral involvement is a necessary feature, those in which it is a frequent feature, those in which it is a rare feature, and the regrettably inevitable iatrogenic group. The author of this section is to be congratulated on providing a selection of excellent photographs to accompany his text. Current investigations are well discussed in relationship to possible underlying aetiological features.

The third section of the book is an excellent review of current concepts of immunological mechanisms in skin disorders, but is perhaps rather out of context, as cutaneous manifestations of diseases with associated rheumatological problems are very briefly dealt with. The volume is to be recommended for all dermatological libraries, and also for the personal bookshelves of those whose imagination is fired by the ever complicated but always stimulating meshwork of connective tissue problems.

RONA M.MACKIE

Human Melanin. EUGENE FOLLMANN (1973) Buenos Aires: Follmann. Pp. 286. Price £4.62.

Professor Follmann is Director of the Investigative Laboratory of the Dermatology Clinic at the University of Beunos Aires, with an East European background of dermatological histopathology. The book he has written is in the form of a justification of his thesis, which is that melanin is a non-specific accretion of the products of combustion of amino-acids and proteins produced by raised intracellular temperature. This interpretation is very largely based on the histopathological appearances of various pigmented lesions which are presented and analysed in great detail. The author is conversant with the current literature and raises some as yet unresolved questions regarding melanogenesis. He falls far short of making the case that: 'No specific melanin producing cells or system exist, but all the cells of the human organism are capable of forming melanin', unless melanin is given an all-embracing, and hence meaningless, definition. Regrettably this seems to be the case and little of value is to be gleaned from the text which abounds in italics, mis-spellings and clumsy constructions.

P.A.RILEY

British Journal of Dermatology (1974) **90**, 117.

Review

THERAPEUTICS XXIV

Drug interaction. III

In a recent survey in general practice, antibiotics were the most frequently prescribed drugs, accounting for 10·6% of all prescriptions (Edwards, 1973), so that the possibility for drug interaction is very great. Increasingly, the beneficial aspects of interactions between antibiotics are being exploited to reduce the emergence of resistant strains of pathogenic organisms, so that detailed knowledge of these is vital. There are, of course, many antibiotics in clinical use, and the groups outlined below cover only those most often used.

Another group of drugs frequently used in hospital practice is the systemic corticosteroids and these are incriminated in many adverse effects. Not the least of these is the activation of underlying infectious processes by reduction of host defence mechanisms, so that it is appropriate to discuss interactions of this group of drugs in the same article.

ANTIBIOTICS

Penicillins (Cloxacillin and Ampicillin)

The absorption of penicillins may be varied in many different ways. Low pH causes decomposition of penicillins though acid stability is very variable, penicillin G being rapidly inactivated and ampicillin remaining relatively unchanged (Goodman & Gilman, 1970a). Antacids, however, and an alkaline medium will reduce the absorption of some penicillins (Hartshorn, 1968a). Neomycin will inhibit the absorption of oral penicillins, but the converse is true of chymotrypsin (Avakian & Kabacoff, 1964).

Once absorbed, the active unbound penicillin can be increased by many drugs which will displace them from protein binding. These include analgesics such as salicylates and phenylbutazone (Hartshorn, 1968a), para-aminobenzoic acid, sulphinpyrazone and sulphonamide (Kunin, 1966). The effect of sulphonamides may, however, vary according to the penicillin and sulphonamide concerned, and in some instances inhibition may occur. Sulphamethoxypyridazine, for example, may lower the total concentration of penicillin, at the same time increasing the *unbound* active *fraction* in both serum and body fluids (Kunin, 1966).

Other antibiotics have many different interactions with the penicillins. As a generalization, those drugs which at normal therapeutic doses are bacteriostatic will inhibit bacteriocidal drugs such as penicillin; however, two bacteriocidal drugs used together are likely to be synergistic and much reduce the emergence of resistant bacteria.

Tetracyclines, erythromycin, streptomycin, kanamycin, chloramphenicol and actinomycin D are amongst the drugs which will inhibit penicillin by diminishing their deleterious effect on bacterial cell walls. Streptomycin, however, may interact synergistically with penicillin in enterococcal infection, as will kanamycin in *Brucella abortus*.

Benzylpenicillin is also synergistic with cephalosporins and erythromycin in pseudomonas infection and penicillinase-producing organisms respectively (Drugs and Therapeutics Bulletin, 1968).

Cloxacillin has an important synergistic action with the broader spectrum penicillins against *Streptococcus pyogenes* and *Staphylococcus aureus* (Sabath & Elder, 1967). Erythromycin, too, whilst generally inhibiting penicillins, will increase their activity against *Staph. aureus* infections (Chang & Weinstein, 1966).

The excretion of penicillin is inhibited by probenecid (Gibaldi & Schwartz, 1968), and this is a useful effect in situations when high blood levels of penicillin are required as in some cases of bacterial endocarditis.

A further important interaction is the cross-sensitivity which occurs with the penicillins and, less frequently known, between penicillin and the cephalosporins (Drug and Therapeutics Bulletin, 1968). Penicillins and heparin used together may be hazardous since the activity of the latter is inhibited and withdrawal of penicillin may result in haemorrhage in patients previously well controlled on heparin (Tuttle, 1969). A further strange interaction is the inhibition of penicillin activity by blue cheese (Tuttle, 1969)!

Tetracyclines

As with the penicillins, the absorption of tetracycline is inhibited by antacid medication (Goodman & Gilman, 1970a), indeed, a number of divalent ions, such as calcium, iron, magnesium, strontium, aluminium and bismuth, form complexes which are relatively poorly absorbed. Sodium bicarbonate will actually reduce absorption by as much as 50%. Foods also reduce absorption, particularly, of course, milk because of the calcium content. Increased absorption of tetracycline may be produced by oral chymotrypsin and citric acid (Kunin, Jones & Finland, 1957). In urinary tract infections acidification of the urine increases tetracycline activity (Kunin & Finland, 1961).

Tetracycline disturbs the alimentary bacterial flora, reducing the synthesis of vitamin K, and therefore enhancing the coumarin anticoagulants. This effect is shared with many of the broad spectrum antibiotics (Klippel & Pitsinger, 1968).

Since tetracyclines occasionally cause liver damage they should be used with caution with other potentially hepatotoxic drugs, and alcohol has been shown to potentiate the antibacterial effect of tetracycline.

The antagonistic effect of tetracyclines to the penicillins has already been mentioned.

Sulphonamides

This group of antibacterial drugs is involved in many interactions with a wide variety of other drugs.

The absorption of sulphonamide is also inhibited by alkalinizing agents and increased by acidification. The latter will decrease the urinary excretion of sulphonamide and reduce its solubility, tending to promote crystalluria (Arita & Hori, 1969).

Mineral oils will inhibit the effects of sulphonamides given for gastro-intestinal infections (Stuart, 1968).

Once in the body, the sulphonamides may be potentiated by drugs causing displacement from plasma proteins. Such drugs include phenylbutazone, oxyphenbutazone and salicylates (Hartshorn, 1968a). Conversely, sulphonamides can enhance the activity of other protein bound drugs by displacement from binding sites. These drugs include the coumarin anticoagulants (which may also be enhanced by depressed vitamin K synthesis mentioned above), the sulphonylurea antidiabetic agents and methotrexate (Hartshorn, 1968a).

Dangerous enhancement of the actions of phenytoin (Hunningshake, 1970) and of other folic acid antagonists may occur (Stuart, 1968).

Sulphonamides are active antibiotics because they compete with para-aminobenzoic acid (PABA) necessary for bacterial replication, thus both PABA and many local anaesthetic agents, such as lignocaine, which share the PABA nucleus, will reduce the effectiveness of sulphonamides (Goodman & Gilman, 1970a). The inhibition of the synthesis of folic acid from PABA may be increased by the addition of trimethoprin which acts on this metabolic pathway at a different point than sulphonamides. This interaction is used in the very effective 'Bactrim' and 'Septrin' preparations.

Sulphonamides also interact with other antibiotics and the different combinations with the penicillins have already been mentioned. Tetracyclines combined with sulphonamides are effective treatment of nocardia, lymphogranuloma venereum and also trachoma (Goodman & Gilman, 1970a).

Griseofulvin

This is an antimicrobial agent frequently used in dermatology and many of its interactions are due to enzyme induction.

The absorption of this drug from the gut is enhanced by fatty foods (Crounse, 1963).

Many drugs acting on the central nervous system interact with griseofulvin. Barbiturates inhibit by enzyme induction, but their depressant effect is enhanced by griseofulvin (Burns & Conney, 1965). This effect may be shared by other sedatives, hypnotics, and orphenadrine (Hartshorn, 1970). Phenothiazines in combination with griseofulvin may induce acute porphyria (Clinico-pathological Conference, 1968).

Antihistamines such as diphenhydramine may result in mutual inhibition given with griseofulvin (Hartshorn, 1968b).

Finally, inhibition of methotrexate occurs with griseofulvin and, perhaps more important, enzyme induction will inhibit the coumarin anticoagulants (Catalano & Cullen, 1966).

CORTICOSTEROIDS

These drugs in many ways are antagonistic to antibiotics since the suppression of inflammatory response allows bacterial penetration through body tissues. This also applies to antiviral and antifungal agents such as idoxuridine and amphotericin B (Stuart, 1968). In severe tuberculous infection, however, systemic corticosteroids may be lifesaving provided adequate antituberculous chemotherapy is provided (Stuart, 1968).

Corticosteroids interact with other hormonal preparations and their analogues. Adrenergic drugs will cause increased intra-ocular pressure and potentiated smooth muscle response in patients taking corticosteroids chronically, and this also applies to anticholinergic therapy. Clearly, this combination is dangerous in glaucoma (Stuart, 1968). The effectiveness of any antidiabetic therapy including insulin is lessened because of the mobilization of tissue glycogen stores by corticosteroids. The anabolic action of growth hormone is inhibited by corticosteroids but all the other actions are increased (Goodman & Gilman, 1970b). The hypercalcaemia induced by parathormone is reduced by systemic corticosteroid therapy. Thyroxine increases body metabolism and, therefore, tissue demand for corticosteroids, and it is mandatory to provide adequate corticosteroid cover in those patients with adrenal hypofunction combined with hypothyroidism, since treatment with thyroxine may provoke acute adrenal crisis.

The problem of increased intra-ocular pressure occurs with other drugs apart from hormones and these include tricyclic antidepressants and the antihistamines. Corticosteroids inhibit nortriptyline metabolism and, therfore, potentiate its action; however, antihistamines reduce corticosteroid breakdown (Stuart, 1968; Hartshorn, 1968a).

Diuretics are sometimes used in patients on corticosteroid therapy and since both drugs tend to cause hypokalaemia, care in potassium replacement is very important, particularly if the patient needs digoxin, since the toxic effects of this drug are increased in the presence of hypokalaemia (Martin, 1971).

Sedatives and hypnotics are enhanced by corticosteroid therapy but the activity of the corticosteroids may be decreased by such drugs as chloral hydrate and barbiturates because of enzyme induction (Hartshorn, 1968).

The activity of oral anticoagulants is reduced by corticosteroids. Nevertheless, severe haemorrhage may be caused by the combination.

Halogenated insecticides, such as DDT, induce the metabolism of cortisol and other corticosteroids, thus reducing their activity (Conney, 1967), but nicotine increases endogenous corticosteroid production and may possibly increase the effectiveness of corticosteroid medication (Martin, 1971).

Analgesics such as phenylbutazone and the salicylates will displace corticosteroids from protein binding allowing increased penetration of tissues and greater activity (Stuart, 1968). Bilirubin, too, will displace corticosteroids (Hartshorn, 1968).

Finally, an obvious and important interaction occurs between live virus vaccines and the corticosteroids and fatalities have occurred because of uninhibited spread of virus (McIver, 1967). Corticosteroid therapy should be discontinued for 3 days before vaccination and not recommenced for 14 days afterwards, though clearly this is not possible in all patients.

Sections of Therapeutics and Dermatology,
Academic Division of Medicine,
The University of Sheffield

I. R. Edwards
S. S. Bleehen

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News and Notices

British Association of Dermatologists Summer Meeting and the Anglo-French Reunion 1974

The Annual Meeting of the British Association of Dermatologists will take place in London on the 3, 4, 5 and 6 July 1974 under the Presidency of Dr Hugh Wallace. The Meeting will be combined with an Anglo-French Reunion, the Anglo-French day being the 5 July. Papers are invited and should be sent by the 1 March 1974 in the form of a summary in triplicate of approximately 200 words, to the Honorary Secretary, Dr J.S. Pegum, The London Hospital, Whitechapel Road, London E1 1BB. The *British Journal of Dermatology* hopes to publish in a Supplement the summaries of those papers which are accepted. These should therefore be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by the *British Journal of Dermatology*.

First Hair Transplant Symposium and Workshop

The American Society for Dermatologic Surgery and The American Academy of Facial Plastic and Reconstructive Surgery, Inc., are co-sponsoring this conference which is designed to offer an opportunity for the exchange of ideas among various disciplines, and to present the latest advances in techniques of hair transplantation. It will be held on 8 and 9 February 1974, at the Stough Dermatology and Cutaneous Surgery Clinic, P.A., Doctors Park, Hot Springs, Arkansas 71901, U.S.A. Attendance will be limited. The speakers will include dermatologists, otolaryngologists and plastic surgeons. For further information, contact D.B. Stough, III, M.D., Program Director (address as above).

Seminar on the Relevance of Animal Modes to Human Dermatopharmacology and Dermatotoxicology

A seminar on the Relevance of Animal Modes to Human Dermatopharmacology and Dermatotoxicology will be held 19–21 September 1974 in the Medical Sciences Building at the University of California, San Francisco, California.

This programme is presented by the Department of Dermatology, University of California School of Medicine, San Francisco, and Continuing Education in Health Sciences, University of California, San Francisco, in co-operation with the American Medical Association Committee on Cutaneous Health and Cosmetics, the Society of Toxicology, The United States Food and Drug Administration, and the United States Army Environmental Hygiene Agency. Partial financial assistance has been provided by Mead Johnson and Company, Evansville, Indiana, and the Society of Toxicology, Richmond, Virginia.

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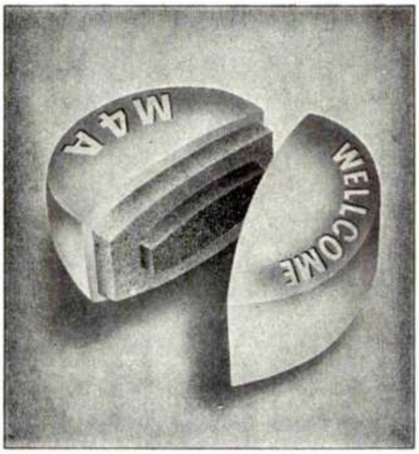

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Textbook of Dermatology

Edited by

Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Robert Harold Champion M.A. M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D.(Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
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William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
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Gunnar Lumbolt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
Stephen Owen Bellamy Roberts M.A. M.B. B.CHIR.(Cantab.) M.R.C.P.(Lond.)
Arthur James Rook M.D.(Cantab.) F.R.C.P.(Lond.)
Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
Peter Derrick Samman M.A. M.D.(Cantab.) F.R.C.P.(Lond.)
Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
Ian Bruce Sneddon M.B. CH.B.(Sheffield) F.R.C.P.(Lond.)
Darrell Sheldon Wilkinson M.D.(Lond.) F.R.C.P.(Lond.)
Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also quarterly reviews of recent advances in contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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40 Meadow Way, Letchworth, Herts.

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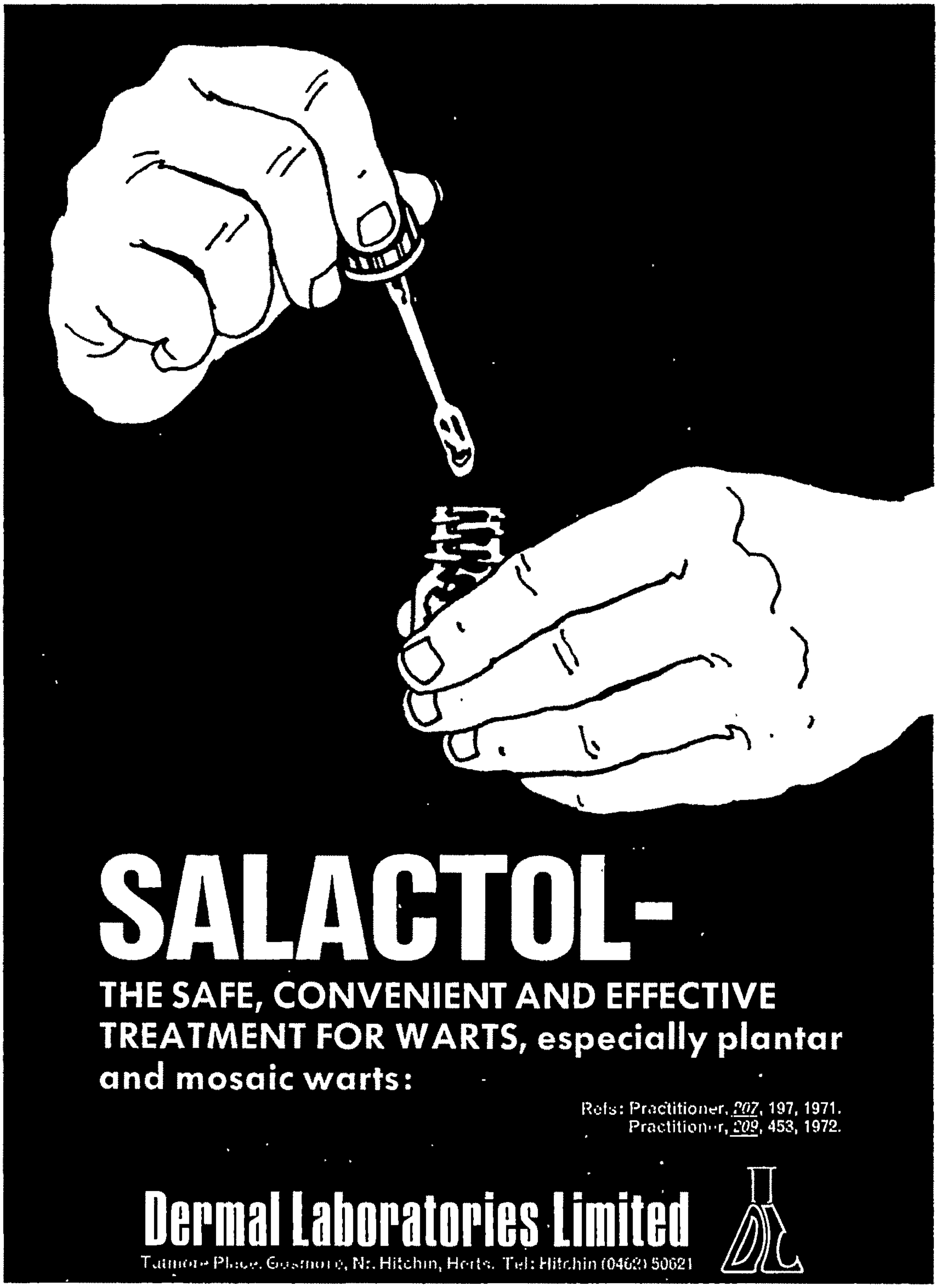

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HAIR TRANSPLANT SURGERY *by* **O'Tar T. Norwood,** *Univ. of Oklahoma, Oklahoma City.* The need for a widely-accepted, accurate and reproducible standard of classification for male pattern baldness has increased with the advent and increasing popularity of hair transplant surgery. The author establishes such a classification and reports its use in determining the incidence of male pattern baldness at various ages in 1,000 Caucasian adult male subjects. Parameters for patient selection are set up and their use demonstrated with various patterns and types of alopecia. In the past eighteen years a number of modifications and refinements have been made in the determination of the approximate size, number and location of grafts for patients before surgery. If properly applied, they can improve the final result considerably. Complications and problems are discussed along with methods for their prevention and correction. Results using only five-millimeter grafts are shown, and specific advantages and indications for the use of five-millimeter grafts are presented. Additional subjects covered include the current status of hair-bearing homografts (co-authored by William T. Summerlin), instruction in placing the hairline, methods of estimating future hair loss, time required and cost of procedure, expectation of the patient, variation in transplanted hairs' color, texture and type, and a survey of hair transplants. *'73, 148 pp. (6 3/4 x 9 3/4), 133 il. (64 in full color), 7 tables, $10.50*

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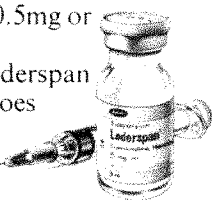

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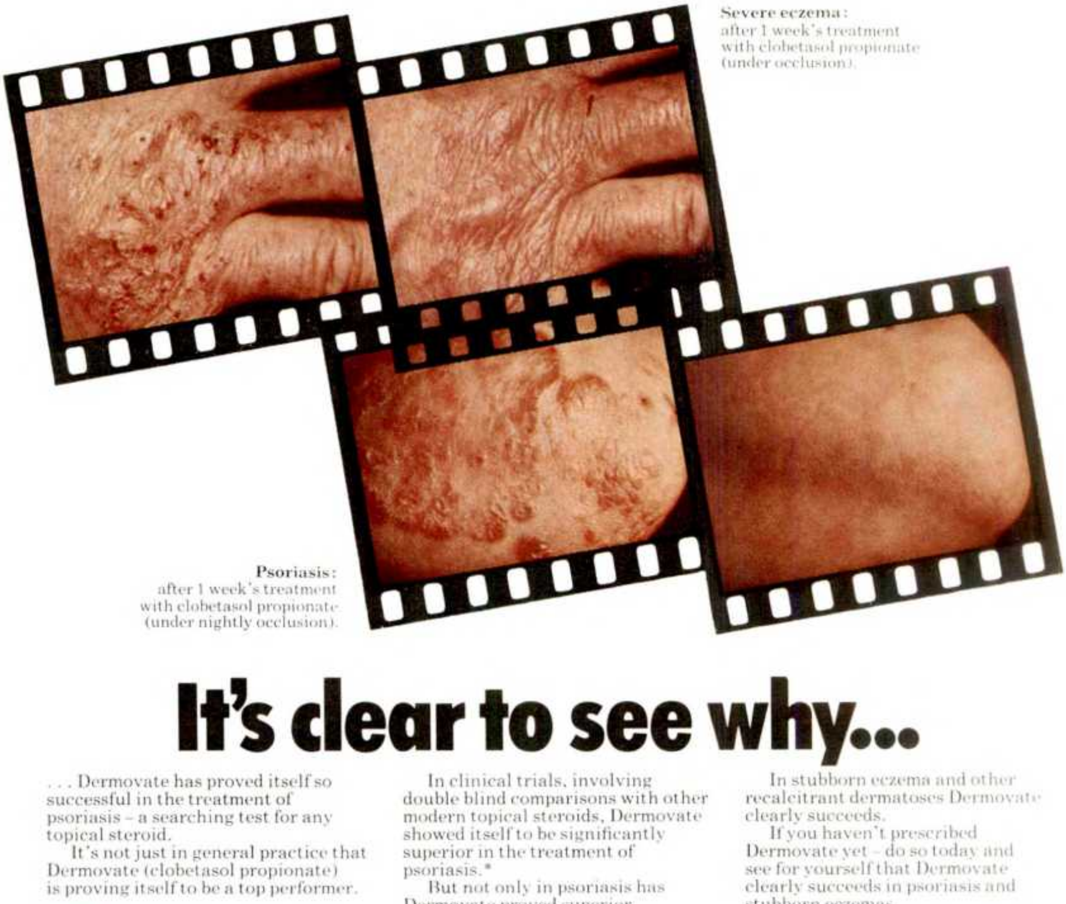

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1. Brit. Med. J. (1967) 4: 535
2. CUTIS (Yorke Medical Group) (1972), 9:77

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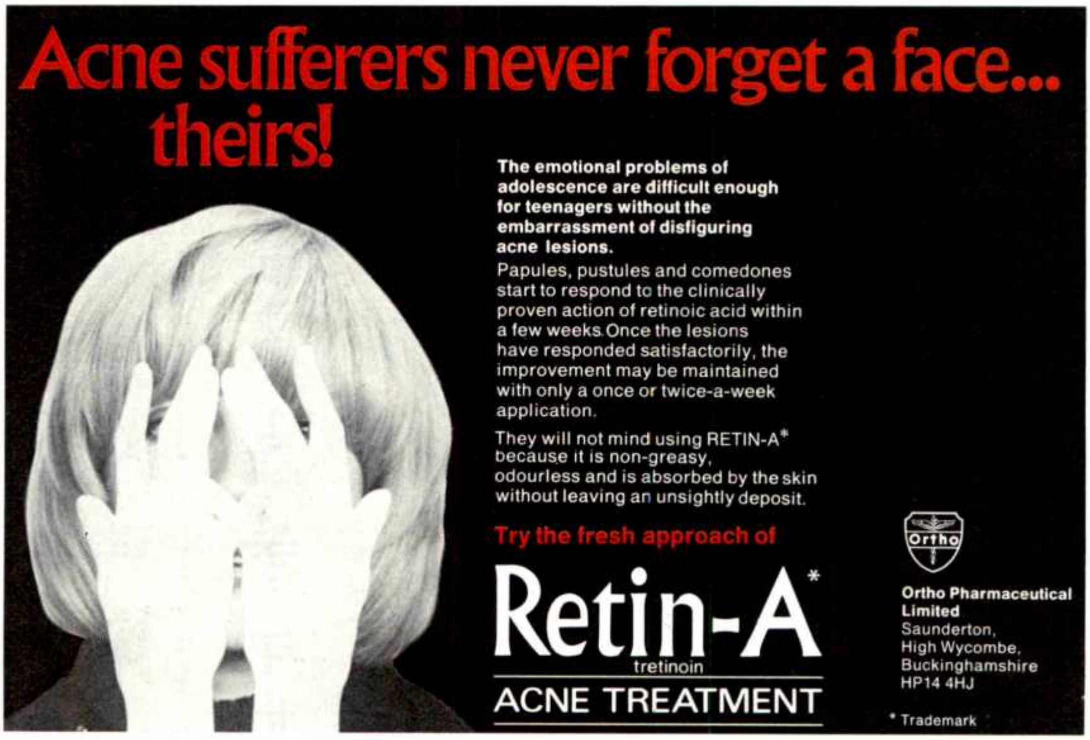

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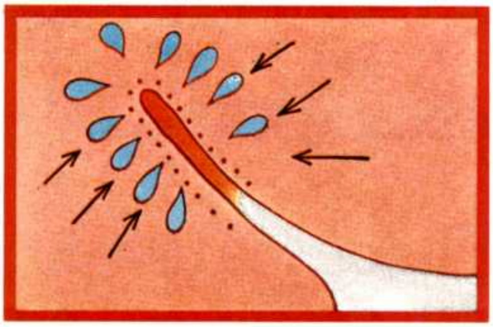
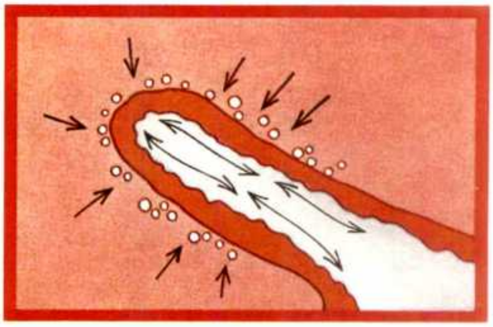
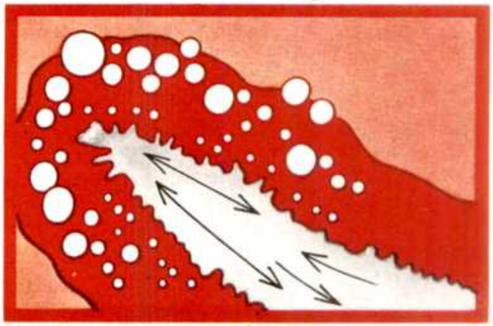


warmth and moisture



maceration and chafing

secondary infection



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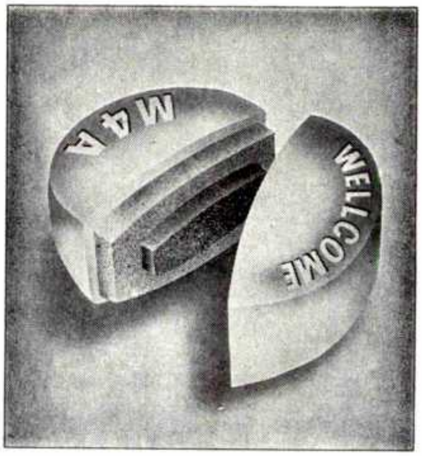
†*Eur J clin Pharmacol* (1971), **3**, 215.



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Clinical and Laboratory Investigations

An immunohistopathological study on the synthesis of immunoglobulins and complement in normal and pathological skin and the adjacent mucous membranes

R.F.M.LAI A FAT, R.H.CORMANE AND R.VAN FURTH

Department of Dermatology, University Hospital Leiden, Department of Dermatology,
University of Amsterdam, and Department of Microbial Diseases, University Hospital,
Leiden, The Netherlands

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SUMMARY

To visualize the cells in skin and mucous membranes in which synthesis of immunoglobulins and complement had been demonstrated, the direct and indirect immunofluorescence techniques were applied, using a fluorescence microscope with epi-illumination, and blue narrow-band excitation light.

Ig-positive cells were found in the lesional skin of patients with discoid lupus erythematosus, (bullous) pemphigoid, dermatitis herpetiformis, mycosis fungoides, atopic dermatitis, and lichen planus, and in the normal mucous membranes deriving from the conjunctiva, the nasal septum, the oral cavity and vagina, but not in the normal skin. The lesional skin showed mainly IgG-positive cells, and the mucous membranes generally had IgG- and IgA-positive cells. In the mucous membranes, with the exception of the oral mucosa, there was a predominance of IgA-positive cells.

The Ig-positive cells were morphologically plasma cells and lymphocytes, and occurred not in groups but scattered in the dermis of the skin and in the lamina propria of the mucous membranes. These cells could be distinguished rather easily from eosinophilic granulocytes, which showed non-specific fluorescence.

The results of the immunofluorescence study were in good agreement with those obtained with the tissue culture technique.

Complement-positive cells could not be demonstrated with the polyvalent antiserum used. A possible explanation for this failure is discussed.

A previous study, in which a culture technique was used to demonstrate the synthesis of immunoglobulins and complement in tissues, showed that normal skin does not synthesize immunoglobulins and that the lesions of various skin diseases not only produce these proteins (IgG, IgA, IgM and IgE) but do so in different patterns, as do the mucous membranes adjacent to the skin; complement (C_3)

synthesis was found in normal skin, and mucous membranes, and in lesions of various skin diseases (Lai A Fat, Suurmond and van Furth, 1973). On the basis of these findings it was of interest to know which cells are responsible for producing these proteins.

It is well known that immunoglobulins are produced by plasma cells and lymphocytes (Gowans & McGregor, 1965; van Furth, Schuit & Hijmans, 1966a, 1966b, 1966c; Chessin *et al.*, 1968; van Furth, 1969; Hijmans, Schuit & Hulsing-Hesselink, 1971; Hijmans & Schuit, 1972) and not by other types of cell. Various complement components are, however, synthesized by macrophages (Stecher, 1970; Schultz, 1971; Ruddy, Gigli & Austen, 1972; van Furth, 1973), and it seemed likely that these components are also produced by other cells. Since it was conceivable that the same cell lines produce immunoglobulins and complement in the skin and mucous membranes, a study was performed with the immunofluorescence technique. Reliable visualization of Ig-positive cells can now be achieved with a fluorescence microscope equipped with a vertical illuminator (Ploem, 1967) using blue narrow-band excitation light, which gives optimal image contrast.

The present investigations had three aims: to demonstrate the cells synthesizing immunoglobulins and complement, to localize these cells, and to compare the results of the immunofluorescence study with those obtained with the culture technique.

MATERIALS AND METHODS

Tissues

Skin samples for the immunofluorescence, histological and culture studies were taken with a biopsy punch (3–5 mm) from the same skin lesion, which was chosen as being in a stage representative for the disease, or, if this was not possible, from two similar lesions. Material from mucous membranes was obtained for these studies as described elsewhere (Lai A Fat *et al.*, 1973).

Immunofluorescence analysis could not be performed in the first part of the investigation because of dessication of the biopsy specimens. To avoid this problem special, storage conditions were applied (see below).

Conjugates and antisera

The conjugates (all purchased from Nordic Immunological Laboratories, Tilburg), with their characteristics and optimal dilution, are shown in Table 1; these data were determined by Dr S. Ashgar, Department of Dermatology, University of Amsterdam. In this Table the abbreviation FTC is used instead of FITC (fluorescein isothiocyanate), because the former reflects the transformation of the isocyanate group into a thiocarbamyl radical.

According to the manufacturer, the monospecificity of the conjugates had been tested first by immunoelectrophoresis and the double diffusion test in agar, using as antigens purified immunoglobulins and Bence Jones proteins of both types and then by the method of van Furth *et al.* (1966d) and Hijmans, Schuit & Klein (1969), using bone marrow samples obtained from patients suffering from different types of paraproteinaemia (Snoijink, 1973).

The following antisera were used: swine anti-human IgD (SwAHu/IgD, batch 1–668), swine anti-human IgE (SwAHu/IgE, batch 770), both obtained from Nordic Immunological Laboratories, Tilburg, The Netherlands, and rabbit anti-human complement serum containing antibodies against C1q, the C3 fragments B_1A and α_2D, and also against $C4 \equiv B_1E$ and $C5 \equiv B_1F$ (batch KH 13-04-A01, Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam). The optimal working dilution for the anti-IgE serum proved to be 1:32, and for the anti-complement serum 1:64 or 1:128.

TABLE 1. Characteristics of the conjugates

Conjugate	FTC content (μg/ml)	Protein content (mg/ml)	Degree of conjugation		Antibody[†] titre	Dilution
			F/P ratio[*] (μg/mg)	Molar ratio (F/P × 0·411)		
Rabbit anti-human IgG/FTC (RAHuIgG/FTC, batch 871)	17·3	12·6	1·37	0·56	4	1:40 1:60
Goat anti-human IgA/FTC (GAHuIgA/FTC, batch 6–171)	31·04	32·8	0·95	0·39	1	1:16 1:32
Goat anti-human IgM/FTC (GAHuIgM/FTC, batch 7–171)	88	51·9	1·7	0·7	4	1:32
Rabbit anti-swine/FTC (RASw/FTC, batch 2–969)	18·3	15·6	1·17	0·48	–	1:32
Goat anti-rabbit/FTC (GAR/FTC, batch 3–171)	60	26·8	2·24	0·92	–	1:64 1:128

[*] Ratio of FTC content to protein content. [†] Determined according to Beutner, Sepulveda & Barnett (1968).

Preparation of sections

The technique currently used in the immunohistochemical laboratory, which has been described by Cormane, Szabó & Hauge (1970), is briefly as follows. The biopsy sample is brought onto the inner wall of a capsule which is placed on the bottom of a small tin. After the capsule has been filled with a 10% gelatin solution (Difco Laboratories, Detroit, Michigan, U.S.A.), the tin and its contents are frozen in liquid nitrogen and stored at −70°C.

Direct immunofluorescence method

Sections were cut 4 μm thick in a cryostat (International Equipment Co., Washington D.C., U.S.A.), placed on a microscope slide, affixed by drying for 30 min, rinsed for 30 min in twice renewed phosphate-buffered saline (PBS) (0·01 M; pH 7·2), incubated with the conjugate in a humid chamber for 30 min, and rinsed again with twice renewed PBS for 30 min.

Indirect immunofluorescence method

This method was used to determine the cellular localization of IgD, IgE, and complement, because no FITC-labelled anti-sera against these proteins were commercially available. The sections were rinsed with PBS for 30 min, incubated with the antiserum for 30 min and rinsed again with PBS as described above. For the second step the sections were incubated for 30 min with one of two conjugates, i.e. rabbit anti-swine (RASw/FTC) or goat anti-rabbit (GAR/FTC), and then rinsed with PBS.

Microscopy

The sections were examined with incident illumination. The microscopic equipment consisted of a Leitz 'Ortholux' fluorescence microscope with a vertical illuminator, after Ploem (1967). The light source was a Xenon XBO 75 W high pressure lamp. As excitation filter a combination of BG 38 (5 mm), KP 490 (2 mm), and GG 475 (2 mm) was used and as barrier filter OG 515 (3 mm). The objectives were Leitz water immersion objectives (× 50 and × 90). Photographs were taken with a Leitz-Orthomat camera and Kodak Tri-X-Pan (27 DIN) film.

Specificity tests

The specificity of the immunofluorescence staining was determined with a FITC-conjugated normal rabbit serum and with the heterologous conjugate (GAR/FTC) in the direct immunofluorescence method. In appropriate cases the specificity was also determined by the blocking test, in which the section is incubated first with a non-conjugated monospecific antiserum and thereafter with a conjugated antiserum of the same specificity.

Examination of the stained sections

After investigation of the epidermis, the whole dermis was examined for the presence of Ig- or complement-positive cells. At least two, and at most ten sections stained with each conjugate were examined.

The specifically positive fluorescing cells were graded as follows: (+) = one strongly positive cell and/or several weakly positive cells; +, = several strongly positive cells; + + = many strongly positive cells.

Histology

Histological analysis was performed on sections of formaldehyde-fixed tissues. The sections were stained with haematoxylin and eosin.

TABLE 2. Results of immunofluorescence and autoradiographic investigation of normal skin and normal mucous membranes*

Origin of biopsy specimens	No. of biopsy specimens	Immunofluorescence†					Autoradiography†				
		IgG	IgA	IgM	IgD	IgE	IgG	IgA	IgM	IgD	IgE
Normal skin	3	–	–	–	–	–	–	–	–	–	–
		–	–	–	–	–	–	–	–	–	–
		–	–	–	–	–	–	–	–	–	–
Mucous membranes											
Conjunctiva	3	+	+ +	–	–		+ + +	+ + +	–	–	–
		+ +	+ +	+	–		+	+ +	–	–	–
		(+)	+	+	–						
Nasal septum	3	+	+ +	+	+	–					
		+	+ +	+	+ +	–	(+)	+ +	(+)	–	–
		+	+ +	–	–	–	+ +	+ +	–	–	–
Oral cavity	5	+	+	–	–						
		(+)	–	–	–		+	–	–	–	–
		+	+	–	–		+	(+)	–	–	–
		+	+	–	–		+	(+)	–	–	–
		(+)	+	–	–		+	(+)	–	–	–
Vagina	4	(+)	+	+	–	–	(+)	(+)	–	–	–
		(+)	+	–	–		+	(+)	–	–	–
		(+)	+	–	–		(+)	(+)	–	–	–
		–	+	–	–		(+)	–	–	–	–

* Open spaces, no immunofluorescence or autoradiographic studies done.

† The specifically fluorescing cells were graded as follows: (+) = one strongly positive cell and/or several weakly positive cells; + = several strongly positive cells; + + = many strongly positive cells.

† The intensity of the autoradiographic lines is classified from: – = negative; (+) = just visible; + = clearly visible; to a maximum of + + + +.

Cultivation of tissue

The method used for the study of immunoglobulin and complement synthesis *in vitro* has been described in a previous paper (Lai A Fat *et al.*, 1973).

TABLE 3. Results of immunofluorescence and autoradiographic analysis of lesional skin of patients with various skin diseases*

Diagnosis	No. of biopsy specimens	Immunofluorescence*					Autoradiography*				
		IgG	IgA	IgM	IgD	IgE	IgG	IgA	IgM	IgD	IgE
Immunological skin diseases											
Discoid lupus erythematosus	6	(+)	+	+			(+)	(+)	−	−	−
		+	−	−			(+)	−	−	−	−
		−	−	−			(+)	−	−	−	−
		−	−	−			−	−	−	−	−
		−	−	−			(+)	−	−	−	−
		−	−	−			(+)	−	−	−	−
(Bullous) pemphigoid	2	+	−	−	−	−	(+)	−	−	−	−
		−	−	−	−	−					
Dermatitis herpetiformis	3	−	−	−	−	−	−	−	−	−	−
		(+)	−	−			(+)	−	−	−	−
		+	−	−	−	+	(+)	−	−	−	−
Malignant reticuloses											
Mycosis fungoides	7	(+)	+	−	−		++	+	−	−	−
		(+)	−	−			(+)	−	−	−	−
		(+)	+	−	−		+	(+)	−	−	−
		(+)	(+)	−	−		(+)	(+)	−	−	−
		(+)	−	−	−		(+)	−	−	−	−
		(+)	+	−	−		(+)	−	−	−	−
		(+)	+	−	−		++	+	−	−	−
Other forms	2	−	−	−	−		−	−	−	−	−
		(+)	−	+	−		(+)	−	(+)	−	−
Miscellaneous group											
Eczemas	4	−	−	−	−		−	−	−	−	−
		−	−	−	−		−	−	−	−	−
		+	+	−	−	−	+	(+)	−	−	−
		+	−	−	−	+	(+)	−	−	−	−
Psoriasis vulgaris	2	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−
Lichen planus	2	(+)	−	−	−		(+)	−	−	−	
		(+)	+	−	−		(+)	−	−	−	
Allergic skin reactions											
Allergic-positive patch test	2	−	−	−	−	−	−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−
Positive tuberculin test	1	(+)	−	−	−	−	(+)	−	−	−	−
Drug eruption	3	−	−	−	−	−	−	−	−	−	−
		−	−	−			−	−	−	−	−
		−	−	−	−	−	−	−	−	−	−

* See Table 2.

RESULTS

The results of this study are presented in Tables 2 and 3, where the tissue culture results are also given.

Normal skin

The histological sections showed occasional lymphocytes, plasma cells, and histiocytes. Ig-positive cells were not found.

The cultures did not synthesize immunoglobulins.

Normal mucous membranes

Histologically, the lamina propria of the mucous membranes (i.e. conjunctiva and nasal, oral, and vaginal mucosae) showed some scattered infiltrations of lymphocytes, plasma cells, and histiocytes, as well as some granulocytes including the eosinophilic type.

In the immunofluorescence study, IgG- and IgA-positive cells were consistently found (Figs. 1 and 2). Cells positive for IgM were sometimes seen in the conjunctiva and the nasal and vaginal mucosae but not in the oral mucosa. The nasal mucosa in two individuals showed IgD-positive cells, but not the oral or vaginal mucosa or the conjunctiva. Cells positive for IgE could not be demonstrated in any of the mucous membranes investigated (Table 2).

With the exception of the oral mucosa, there was a preponderance of IgA-positive cells in all the mucous membranes investigated. A notable finding was the very distinct and specific fluorescence seen at the surface of the epithelium of the conjunctiva stained with the anti-IgA conjugate. This specific fluorescence was caused by the IgA in lacrimal fluid, in which it is the predominant class of Ig (Chodirker & Tomasi, 1963; Josephson & Weiner, 1968; Little, Centifanto & Kaufman, 1969). With the anti-IgG and anti-IgM conjugates, weak fluorescence was seen at the surface of the conjunctiva, but none was observed with the anti-IgD conjugate.

The results of the individual cultures of mucous membranes showed IgG and IgA synthesis, and in one case IgM synthesis.

Pathological skin

The histological findings in the biopsy specimens were in accordance with the clinical diagnosis.

The results of the immunofluorescence study of the various skin diseases are given in Table 3. In general, IgG- and IgA-positive cells were demonstrated in tissues known to produce these immunoglobulins (Figs. 3, 4 and 5). A few cases showed IgA-positive cells (e.g. one patient with mycosis fungoides and another with lichen planus) although the cultures were negative in this respect. The section of one patient with discoid lupus erythematosus showed IgM-positive cells, but the culture did not synthesize this protein; in this patient the biopsy specimen used for the immunofluorescence analysis had been taken from another lesion. IgE-positive cells were found in one patient with dermatitis herpetiformis and in another with atopic dermatitis (Fig. 3c), but no IgE synthesis could be demonstrated with the culture technique.

The results of the individual cultures of pathological skin showed, in general, IgG synthesis and sometimes also IgA synthesis. Only one culture synthesized IgM.

Morphology of the Ig-positive cells

The Ig-positive cells found in the mucous membranes and in the lesional skin had the morphological features of plasma cells (with an eccentric nucleus) and lymphocytes which have a centrally located nucleus surrounded by a small rim of cytoplasm (Figs. 3b and 5). The fluorescence of the IgG-positive cells was generally less conspicuous than that of the other Ig-positive cells. All of these positive cells had a homogeneous fluorescence pattern.

The positive plasma cells and lymphocytes were scattered in the dermis; groups of these cells were not seen.

The positive plasma cells and lymphocytes could be distinguished rather easily from another type

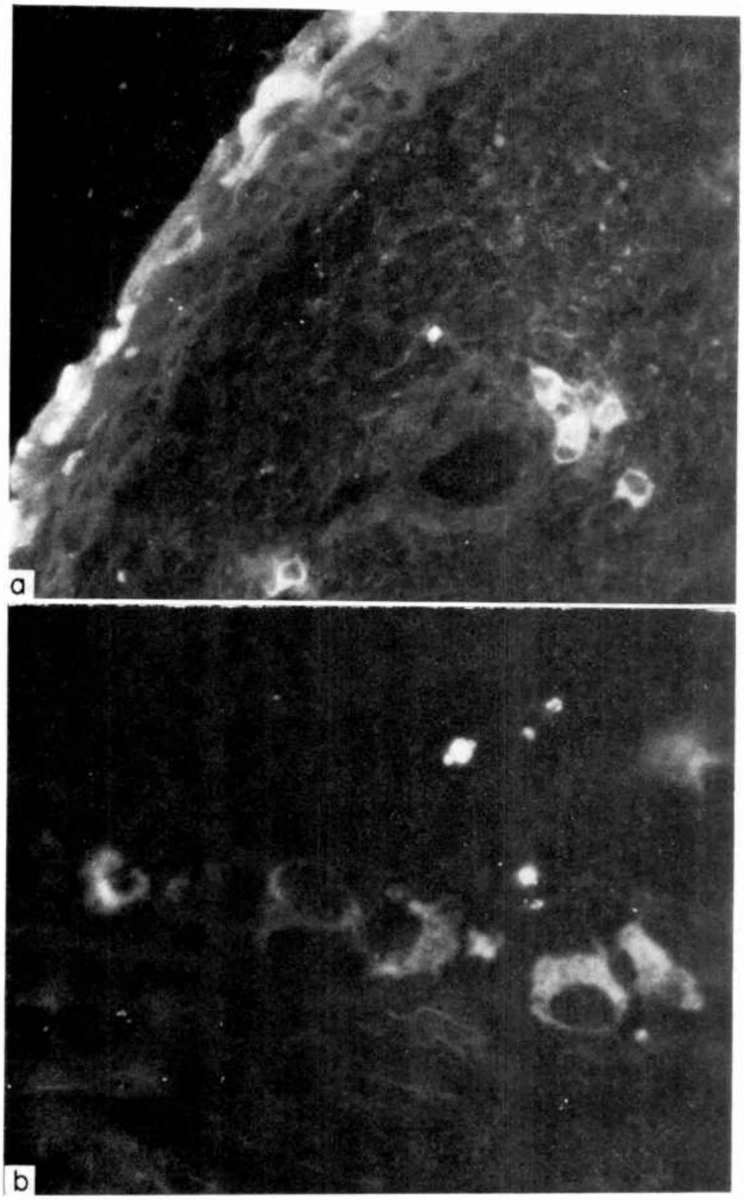

FIGURE 1. Conjunctiva. (a) IgA-positive plasma cells and lymphocytes in lamina propria. Note strong fluorescence at the surface of the epithelium (× 450). (b) IgA-positive plasma cells and lymphocytes in lamina propria (× 900).

of cell showing granular fluorescence and frequently a bilobed nucleus (Fig. 4c). The distinct granular fluorescence of these cells was by no means specific, because they were also stained by an unrelated (heterologous) conjugate (GAR/FTC). They also showed a strong yellowish-brown autofluorescence and were found mainly in sections from patients with (bullous) pemphigoid, dermatitis herpetiformis, mycosis fungoides, and positive allergic patch tests to dinitrochlorobenzene. Indications that these cells were eosinophilic granulocytes were supported by the fact that they stained with eosin both in

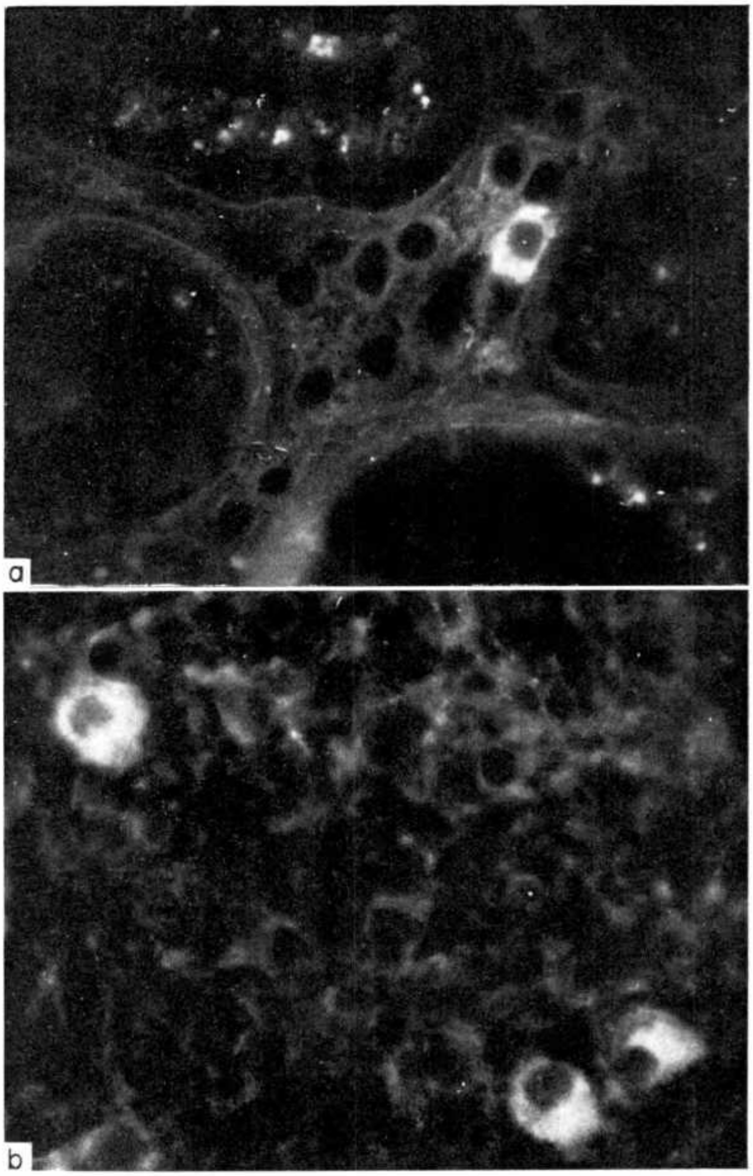

FIGURE 2. Nasal mucosa. (a) IgA-positive plasma cell between acini. At the top left, there is a secretory duct with granular fluorescence in the apical part of the cell. (b) IgG-positive plasma cells (× 900).

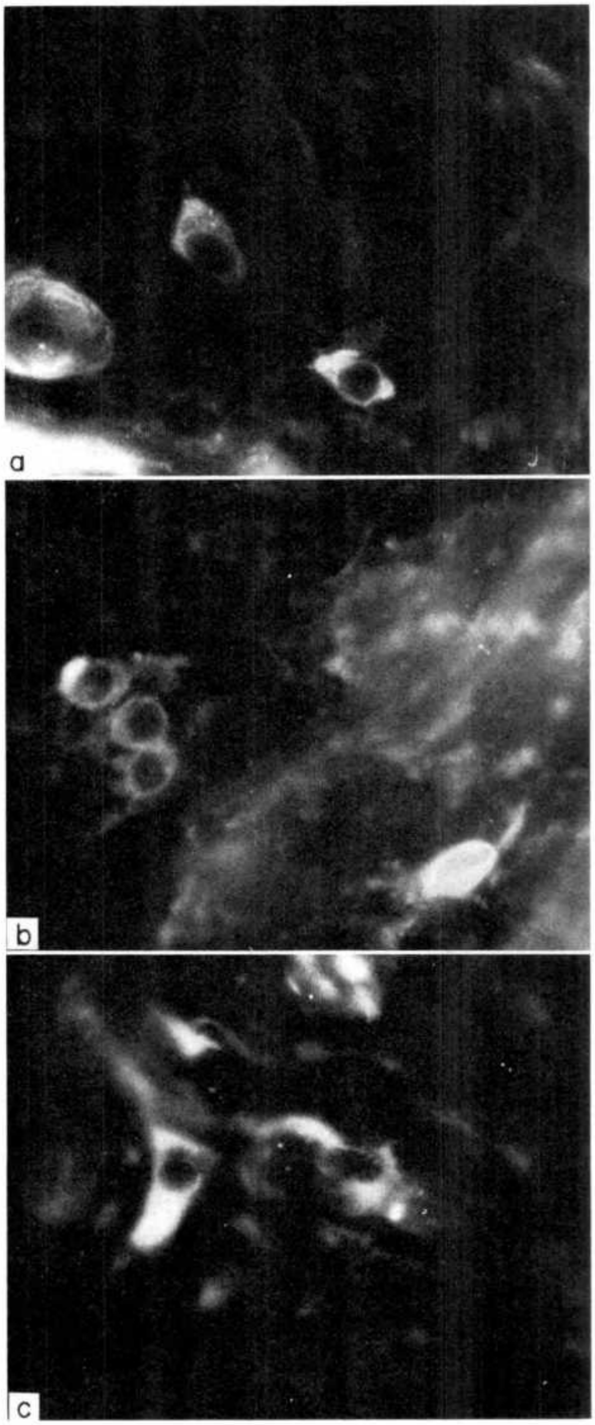

FIGURE 3. Lesional skin of patient with atopic dermatitis. (a) IgG-positive plasma cells. (b) IgA-positive lymphocytes. (c) IgE-positive plasma cell (×900).

the sections treated with the conjugates and then washed with phosphate-buffered saline, and in histological sections.

Complement-positive cells

With the polyvalent antiserum used, cells positive for complement could not be demonstrated in normal or pathological skin or in normal mucous membranes.

Specificity tests

After staining the sections with the FITC-labelled normal rabbit serum or with the heterologous conjugate (GAR/FTC), and after the blocking test, no Ig-positive plasma cells or lymphocytes were found.

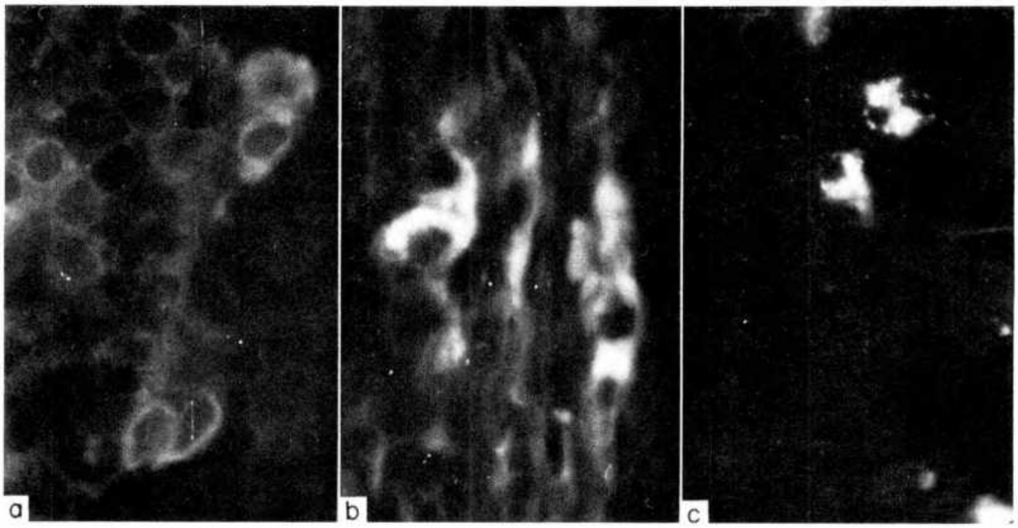

FIGURE 4. Lesional skin of patient with mycosis fungoides. (a) Weakly IgG-positive lymphocytes. (b) IgA-positive plasma cells. (c) Eosinophils non-specifically stained with anti-IgA conjugate; the cell at the top has a bilobed nucleus (× 810).

DISCUSSION

The results obtained with the immunofluorescence technique correspond well with those obtained with the tissue culture method (Tables 2 and 3). When immunoglobulin synthesis was found with the tissue culture technique, Ig-positive plasma cells and lymphocytes could be demonstrated in the biopsy specimen of the same lesion. This good correlation between the occurrence of synthesis and the presence of Ig-positive cells has also been found for lymphoid organs and for lymphocytes in the circulation (van Furth *et al.*, 1966a, 1966b, 1966c; van Furth, 1969).

In a number of cases (e.g. the immunological skin diseases), a small amount of IgG synthesis was demonstrated with the tissue culture technique, but the cells producing this protein could not be found. For the anti-IgG conjugate, this discrepancy is most probably attributable to a weak image contrast due to unwanted background staining caused by the IgG in tissue fluid. Attempts to reduce this background staining by fixing the sections with absolute methyl alcohol, acetone, or an acetic acid–ethanol solution (5% glacial acetic acid in 96% ethanol) prior to incubation with this conjugate, failed. Because of this background fluorescence, the IgG-positive cells were less conspicuous than the other positive cells. Another possibility is that these cells were missed because only a limited number of sections were examined.

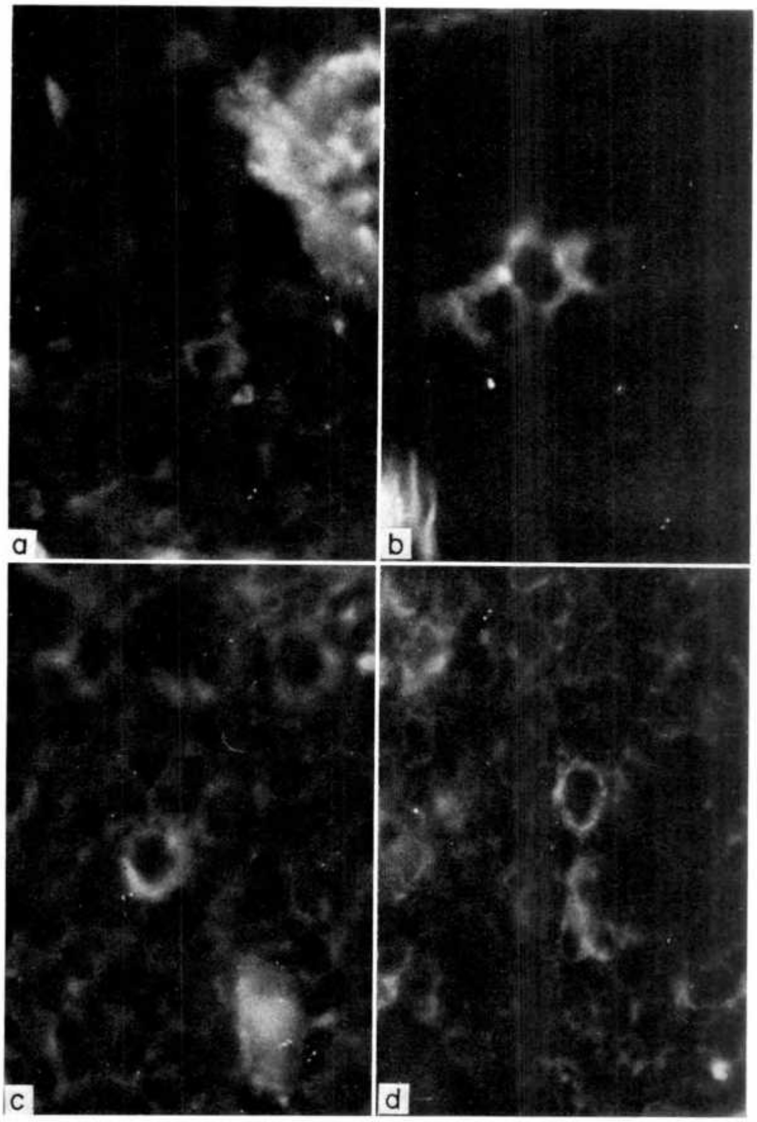

FIGURE 5. Lesional skin of patients with various skin diseases. (a) Discoid lupus erythematosus. Weakly IgG-positive lymphocyte in the infiltrate. (b) (Bullous) pemphigoid. IgG-positive lymphocytes. (c) Brill–Symmers disease. IgG-positive lymphocyte and (d) IgM-positive lymphocyte (× 900).

In a few cases, IgA- and IgM-positive plasma cells and/or lymphocytes were demonstrated, but synthesis of these proteins was not found in the culture study. In these cases the specimens may have contained only a few Ig-producing cells whose production was below the level of detection.

Except in the oral mucosa, IgA-positive cells were more numerous than IgG-positive cells in the mucous membranes. The findings in the nasal mucosa are in accordance with those of Brandtzaeg, Fjellanger & Gjeruldsen (1967). The oral mucosa, however, usually showed equal numbers of IgG- and IgA-positive cells, in contrast to the findings of Brandtzaeg & Kraus (1965) and Brandtzaeg *et al.* (1967), who reported a preponderance of IgG-positive cells. This difference might be explained by the weak image contrast already referred to, which may have caused us to miss IgG-positive cells. Unlike Tourville *et al.* (1970), we were able to demonstrate IgG- and IgA-positive and sometimes IgM-positive plasma cells and lymphocytes in the vaginal mucosa.

The lamina propria of the mucous membranes and the dermis of the skin generally showed scattered Ig-positive plasma cells and lymphocytes. A satisfactory explanation for this localization cannot be given, but it suggests that the Ig-synthesizing cells may originate from lymphoid organs and reach the skin via the blood.

The same localization of Ig-positive cells in the lamina propria has been found in the respiratory, digestive, and female genital tracts (Brandtzaeg & Kraus, 1965; Crabbé and Heremans, 1966; Eidelman *et al.* 1966; Brandtzaeg *et al.*, 1967; Heremans & Crabbé, 1967; Gelzayd, Kraft & Fitch, 1967; Gelzayd, Kraft & Kirsner, 1968; Martinez-Tello, Braun & Blanc, 1968; Rossen *et al.*, 1968; Söltoft, 1969; Tourville *et al.*, 1969, 1970; Savilahti, 1972).

In lesional skin of patients with lupus erythematosus, pemphigus, (bullous) pemphigoid, dermatitis herpetiformis, and also in those with mycosis fungoides (to be published), extracellularly located immunoglobulins and complement were demonstrated with the immunofluorescence technique. The results of the culture and immunofluorescence studies make it very likely that some of these extracellularly located proteins in the lesional skin are produced locally. The function of the locally synthesized immunoglobulins in skin disease, i.e. their antibody activity, has not been investigated yet. The possibility that these immunoglobulins are antibodies directed against such antigens as paramyxovirus-like structures, mycosis cells, (altered) basement membrane, and the intercellular substance of the epidermis, has still to be investigated.

The cellular localization of C3 synthesis in normal skin, normal mucous membranes and the lesions of various skin diseases could not be demonstrated. This failure may have been due to the strong background staining of the tissue caused by the reaction with collagen of anti-C1q antibodies (present in the antiserum against complement), as it has antigenic determinants in common with C1q (Müller-Eberhard, 1971). It therefore seems likely that FITC-labelled antiserum directed against a single complement component (e.g. C3) will serve more appropriately for the detection of complement-producing cells.

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Dermatitis herpetiformis: an evaluation of diagnostic criteria

LIONEL FRY AND P.P.SEAH

Department of Dermatology, St Mary's Hospital, London, W.2

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SUMMARY

Forty-two patients in whom a clinical diagnosis of dermatitis herpetiformis (DH) had been made, were studied. All patients had a small intestinal biopsy, a biopsy of uninvolved skin for detection of the presence of IgA deposits by immunofluorescence, serum and red cell folate estimations and examination of the serum for anti-reticulin antibody. The response of the skin eruption to dapsone was noted. Thirty patients had biopsies of skin lesions for routine histological examination.

IgA deposits were found in the uninvolved skin of thirty-five of the forty-two patients. Evidence of an enteropathy was found in thirty-four of those thirty-five patients. Of the seven patients who did not have IgA in the uninvolved skin, only one showed evidence of enteropathy, which was slight, and this patient was shown subsequently to have pemphigus and not DH. Of the thirty biopsies of skin lesions, only five showed all the features usually considered to be characteristic of DH. Anti-reticulin antibody was found in eight patients, and in all of these IgA was present in the uninvolved skin. Low red cell folate levels were found in eleven patients (all of whom had an enteropathy and IgA in the uninvolved skin). Low serum folate levels were found in thirty-four patients, thirty-one of whom had IgA deposits in the uninvolved skin and an enteropathy; in the other three patients who did not have IgA deposits or an enteropathy, all were on dapsone and this may have accounted for the abnormal result.

In forty-one of the forty-two patients the rash was dapsone responsive. The patient who showed no response to dapsone had no IgA in the uninvolved skin and no evidence of enteropathy. Follow-up showed that of the seven patients with no IgA in the skin, one had pemphigus, and in three the rash eventually cleared spontaneously and the patients no longer required treatment. There was no evidence of spontaneous remissions in the patients with IgA in the uninvolved skin.

It would appear that the presence of IgA deposits in the uninvolved skin is part of the syndrome of DH and that the diagnosis should not be made unless these deposits are found.

Dermatitis herpetiformis (DH) has on clinical grounds been known as one of the so-called bullous disorders. The criteria for making the diagnosis of DH and distinguishing it from the other bullous disorders have been arbitrary and empirical. In his review of the diagnosis of DH, Smith (1966) stated that there is no 'universal agreement as to the criteria for making the diagnosis of DH'. Until 1967 it mattered little as to the exactness in diagnosis as the treatment was empirical, and nothing was known of the cause of the disorder. In fact, the commonest way of establishing the diagnosis was, and still is in the majority of dermatological departments, empirically to assess the response of the rash to dapsone or sulphonamides. This is unsatisfactory, as it is now known that some other skin disorders also clear with dapsone.

However, following preliminary reports of an enteropathy in DH (Marks, Shuster & Watson, 1966; Fraser, Murray & Alexander, 1967; Fry *et al.*, 1967), it has been shown that this enteropathy is due to gluten sensitivity (Fry *et al.*, 1967, 1969; Shuster, Watson & Marks, 1968), that all patients with DH have this enteropathy (Fry *et al.*, 1972), and that the skin rash is also gluten dependent (Fry *et al.*, 1968, 1969, 1973). Thus, there is now a specific treatment for patients with DH, namely a gluten-free diet (GFD). Although this is a harmless treatment compared to many others given by the medical profession today, it does impose a very real social handicap on the patients, and it is without question very difficult for a large proportion of the patients to adhere strictly to a GFD. If a GFD is to be successful in the clearing of skin lesions in DH, it must be strict (Fry *et al.*, 1973) and, at the present time, must be adopted for life. Thus, before embarking on this treatment it is most important to establish the correct diagnosis. In our opinion the usual criteria of clinical and histological features, and response to dapsone, are not precise enough in establishing the correct diagnosis in all patients.

A number of immunological abnormalities in patients with DH have recently been described. These include the presence of circulating anti-reticulin (ARA) and anti-nuclear antibodies (Seah *et al.*, 1971a), IgA deposits in the skin (Van der Meer, 1969; Chorzelski *et al.*, 1971; Seah *et al.*, 1972), and circulating immune complexes (Mowbray *et al.*, 1973). A high incidence of folate deficiency (Fry *et al.*, 1967; Brow *et al.*, 1971) and splenic atrophy (Pettit *et al.*, 1972) have also been described. Thus, with a clearer understanding of the cause of DH and a number of the associated pathological abnormalities, we have attempted to assess the most useful criteria for establishing the diagnosis.

PATIENTS AND METHODS

Patients

Forty-two patients (aged 22–75 years), comprising twenty males and twenty-two females, were studied. They were referred with a clinical diagnosis of 'dermatitis herpetiformis' from nine consultant dermatologists over a period of 6 years.

Methods

The patients were assessed by the following seven criteria.

(*a*) *Clinical features.* The clinical features that were present at the first consultation with the referring consultant were noted. The features usually considered to be characteristic of DH are intensely irritating grouped blisters on the knees, elbows, buttocks, scapulae and other areas which may be subjected to pressure. The blisters frequently arise on an urticarial base. Because of the intense irritation only grouped excoriated papules are sometimes seen at the above sites. The rash may therefore be considered to be polymorphic, as originally described by Duhring.

(*b*) *Response of rash to dapsone and sulphapyridine.* The response of the skin rash to dapsone or sulphapyridine was assessed. Initially patients were given 100 mg of dapsone per day; if there was no improvement the dose was increased to 200 or even 300 mg/day. Patients were seen at weekly intervals. Dapsone was the drug of first choice and sulphapyridine was used only when patients could not tolerate dapsone. The dose of sulphapyridine varied from 1 to 3 g/day. In patients whose rash cleared, therapy was withdrawn to see if relapse of the rash occurred.

(*c*) *Histology of skin lesions.* Skin biopsies had been carried out on thirty of the forty-two patients. Vesicles or early erythematous lesions were taken under local anaesthesia and processed for routine histology. An appraisal as to why so few patients had had biopsies indicated that, whilst absence of suitable lesions for biopsy was a factor, this was minimal, and that in the main most of the dermatologists elected to accept the clinical features and response to dapsone and/or sulphapyridine as sufficient evidence for the diagnosis of DH, and not to perform a biopsy.

The histological features were assessed according to the criteria of Pierard & Whimster (1961) and graded according to the histological features considered to be characteristic of DH. These included the presence of sub-epidermal bullae, papillary micro-abscesses and infiltration with neutrophils and eosinophils. For the purpose of this study, biopsies were assessed according to the presence of these features. A biopsy showing all three features was scored + + +, with two + +, with one +, and 0 for none.

(*d*) *Anti-reticulin antibody.* An autoantibody directed against reticulin has been found in patients with DH (Seah *et al.*, 1971a) and in patients with coeliac disease (Seah *et al.*, 1971a, 1971b; Alp & Wright, 1971; Von Essen, Savilahti & Pelkonen, 1972). The presence of this antibody was looked for by indirect immunofluorescence according to the method of Seah *et al.* (1971a). A sheep-anti-human immunoglobulin fluorescein (FITC) conjugated anti-serum (Wellcome Reagents, lot K.4761, F/P ratio: $OD_{495:280}$–0·85, molar 3·7) was used at a working dilution of 1:32.

(*e*) *IgA in skin.* This was detected by direct immunofluorescence as described by Seah *et al.* (1972). Clinically uninvolved skin was taken from the buttocks using a 3 or 4 mm punch biopsy under 2% lignocaine local anaesthesia, and embedded in OCT (Lab. Tek, Indiana) in pre-cooled isopentane. 6 μm sections of skin were cut in a cryostat, transferred onto glass slides and air-dried. The slides were prewashed in phosphate buffered saline (PBS) pH 7·2 and then stained with the anti-IgA fluorescein conjugate for 30 min. After a final wash for 1 h in PBS pH 7·2, they were mounted with buffered glycerol, pH 8·0, and examined on a Riechert 'Diapan' microscope with a quartz–iodine light source, a Balzer FITC-3 primary interference filter and a Kodak Wratten 12 secondary filter. Biopsies were taken irrespective of whether the patient was on therapy (dapsone, sulphapyridine or a gluten-free diet) or not, and in those patients with skin lesions, care was taken to obtain the biopsies from clinically uninvolved areas well away from the lesions. A commercial sheep-anti-human IgA fluorescein (FITC) conjugate (Wellcome Reagents, lot K.5777, F/P ratio: $OD_{495:280}$–0·71, molar 3·3) was used at a dilution of 1:12. Prior testing of the conjugate against α-chain monoclonal bone marrow was positive, and negative results were obtained with γ and μ heavy chain and k and λ light chain monoclonal bone marrow.

Controls were performed using uninvolved skin from normal volunteers, and by staining some of the DH biopsies with sheep-anti-rabbit immunoglobulin fluorescein (FITC) labelled antisera (Wellcome Reagents, Lot K.5863). Blocking experiments were performed using an unconjugated sheep-anti-human IgA antiserum (Wellcome Reagents).

(*f*) *Small intestinal biopsy.* This was performed using a Crosby capsule under radiographic control. Biopsies were taken at the level of the ligament of Trietz. Specimens were examined on a dissecting microscope and then fixed in formol saline and embedded in wax. 7 μm sections were cut and stained with haematoxylin and eosin.

Macroscopic appearance. Under the dissecting microscope, the appearance of the biopsies was classified into four main categories: flat, convoluted, leaves only, and fingers and leaves. Flat and convoluted specimens were considered to be abnormal.

Microscopic appearance and lymphocyte count. At the microscopic level, sections of the jejunal biopsies were examined for evidence of infiltration with lymphocytes. The number of intra-epithelial lymphocytes was quantified in each specimen, as described by Fry *et al.* (1972), as an index of this lymphocytic infiltration, and expressed as intra-epithelial lymphocytes per 1000 epithelial cells. A count above 200 is suggestive of an abnormality. A count above 300 is definitely abnormal.

(*g*) *Folate status.* Serum folate levels were estimated using *Lactobacillus casei* as the test organism (Waters & Mollin, 1961). The normal range is 6·0–21·0 ng/ml. Levels less than 3·0 ng/ml were regarded as abnormal, and those between 3·0 and 5·9 ng/ml as borderline. Red blood cell folate levels were estimated according to the modification of the serum folate assay described by Hoffbrand, Newcombe & Mollin (1966). The normal range is 160–640 ng/ml packed red blood cells.

RESULTS

(*a*) *Clinical features*

Only fifteen (36%) of the forty-two patients had on their first consultation at a dermatological clinic the typical features of dermatitis herpetiformis which enabled the diagnosis to be made with certainty on this occasion.

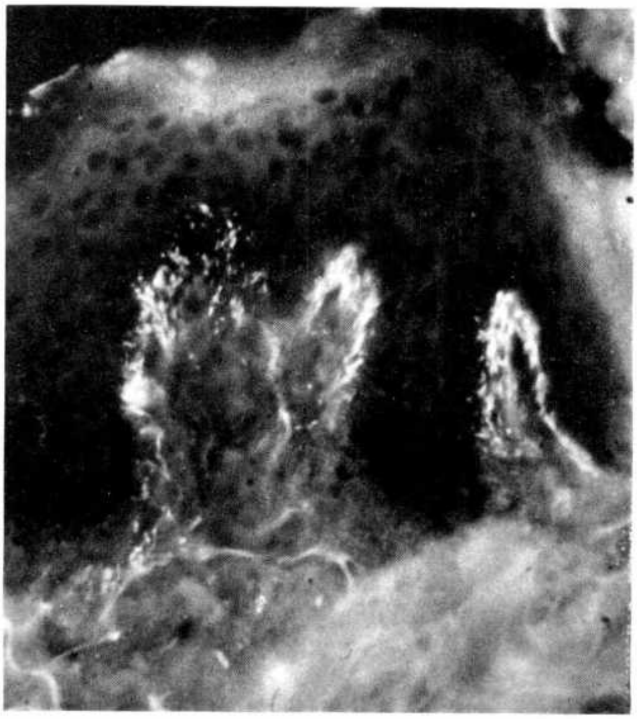

FIGURE 1. Uninvolved DH skin—direct immunofluorescence showing 'papillary' IgA deposits in the dermal papillae (× 400). (By permission of the editor of the *Proceedings of the Royal Society of Medicine.*)

(*b*) *Response of rash to dapsone or sulphapyridine*

The skin lesions improved with dapsone in forty-one of the forty-two patients and the eruption recurred when the drug was withdrawn. Three patients whose rash responded favourably to dapsone were unable to tolerate this drug, and in two of these the eruption was controlled by sulphapyridine.

(*c*) *Histology of skin lesions*

Of the thirty biopsies, five (17%) had all three histological features (+ + +) characteristic of DH, seven (23%) had two (+ +), 16 (53%) had one (+), and two (6%) no features (o).

(*d*) *Anti-reticulin antibody*

This antibody was detected in eight (19%) of the forty-two patients. Seven of these patients were on a normal diet and one on a gluten-free diet.

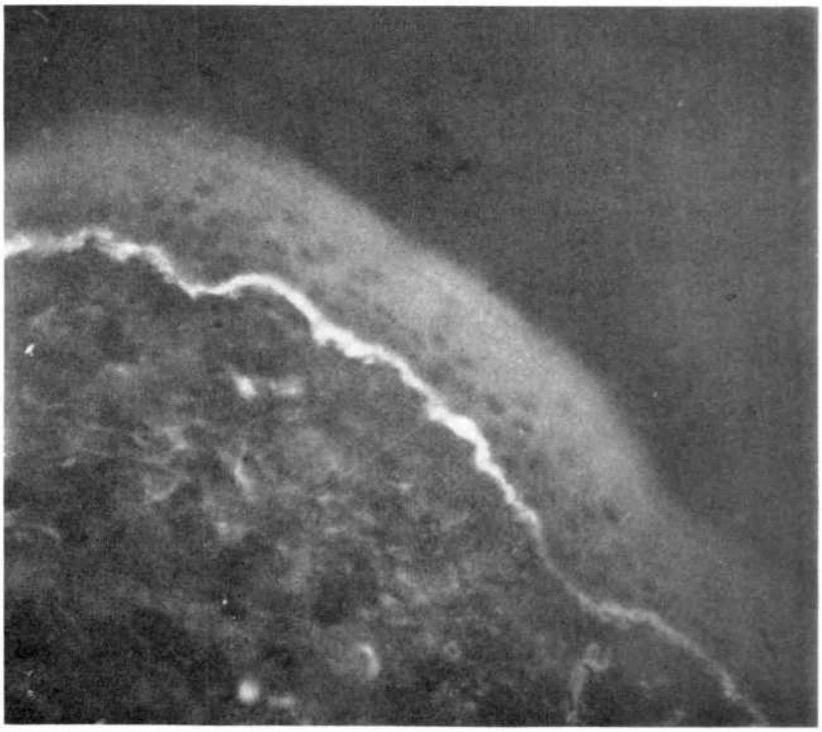

FIGURE 2. Uninvolved DH skin—direct immunofluorescence showing 'continuous' pattern of IgA staining (× 160).

(*e*) *IgA in skin*

IgA deposits were detected in thirty (83%) of the forty-two patients, in the dermis of the uninvolved skin. These deposits were most commonly seen (in thirty-one of the thirty-five) as fibrillar fibres in the tips of the dermal papillae (Fig. 1), and less frequently (in four of the thirty-five patients) as a continuous line (Fig. 2) at the dermo-epidermal junction. No positive immunofluorescence was seen with skin from normals, or with the control fluorescein conjugated anti-serum when tested against the DH skin biopsies. The specificity of the positive IgA staining was substantiated by the ability of unconjugated anti-sera to abolish the positive staining with FITC-conjugated anti-human IgA.

(*f*) *Small intestinal biopsy*

Macroscopic appearance. In the forty-two patients the macroscopic appearance of the small intestine was abnormal in seventeen. It was flat in nine and convoluted in eight. In the remaining twenty-five biopsies, the appearances were leaves only in six, and fingers and leaves in nineteen.

Intestinal epithelial lymphocyte count. The lymphocyte count was below 200 in eight of the forty-two biopsies. Of the nine flat biopsies, all had counts above 200. Of the eight convoluted biopsies, one had a count below 200, being 130. This particular patient had a lymphoma of the small intestine. Of the six biopsies which showed leaves only, one had a count below 200. Of the biopsies showing fingers and leaves, six of the nineteen biopsies had counts below 200.

(*g*) *Folate status*

Serum folate. Thirty-four of the forty-two patients had low serum folate levels. In twenty the level was below 3 ng/ml.

Red blood cell folate. Eleven of the forty-two patients had low red blood cell folate levels.

CORRELATION AND ANALYSIS OF RESULTS

The results show that the incidence of a typical clinical presentation of DH in this series was very low. A study of the notes shows that the diagnosis of DH was considered in the majority of patients only after a number of attendances at the skin clinic. Likewise, a routine biopsy showed all the features characteristic of DH in less than 20% of patients.

The criterion which eventually suggested the diagnosis in forty-one of the forty-two patients was the response of the eruption to dapsone. In the one patient who failed to improve with dapsone, the clinical features were considered compatible with and suggestive of DH. This was a woman of 40 with a 6-year history of grouped, irritating papules on the legs and trunk.

The two objective investigations showing the highest incidence of abnormality were the presence of IgA in the uninvolved skin (thirty-five of the forty-two patients) and evidence of gluten-sensitive enteropathy (thirty-five of the forty-two patients). There was good correlation between these two findings. Of the thirty-five patients with IgA in the skin, thirty-four had evidence of a gluten-sensitive enteropathy demonstrated by an abnormal macroscopic appearance of the mucosa and/or abnormal lymphocytic infiltration of the small intestinal epithelium. The one patient who did not have an abnormal small intestinal mucosa macroscopically or increased lymphocytic infiltration (a count of 89) but had IgA in the uninvolved skin, was a man of 55 with a 10-year history of a widespread irritating vesiculo-papular eruption which responded to dapsone. In view of our finding of one other patient with a normal lymphocyte count in the small intestinal epithelium (130) but a convoluted pattern on biopsy and who had a lymphoma of the small intestine, it is possible that our patient with a count of 89 has a basic abnormality of his lymphocytes, and may also be a candidate for a lymphoma.

Of the seven patients who did not have IgA in the skin, only one had an abnormality of the small intestine, which was slight, manifested by a lymphocyte count of 253. This patient was a woman of 43 with an 11-year history of a blistering eruption controlled by sulphapyridine. On further investigation she was found to have pemphigus substantiated by histological (intra-epidermal blister with acantholysis) and immunological studies (circulating and *in vivo* antibodies bound to the intercellular cement substance of the epidermis).

Thus all but one of our patients with IgA in the skin had definite evidence of an enteropathy, and only one of our patients who did not have IgA in the skin (and who was subsequently shown to have pemphigus) showed an abnormality of the small intestine which was slight. Thus there is nearly 100% correlation between IgA in the skin and evidence of an enteropathy, and these appear to be the best diagnostic criteria for DH at the present time.

In six of the seven patients with no IgA in the uninvolved skin, the rash responded favourably to dapsone in a manner similar to those with IgA in the uninvolved skin. Of those six patients, one has been shown to have pemphigus, but no diagnosis has been made in the other five. However, in three of these five patients, the rash has cleared after intervals of 6 months, 2 years and 5 years respectively. These patients no longer require dapsone. In the past these patients would undoubtedly have been labelled spontaneous remissions of DH, but in our opinion these patients have never had DH. None of our patients with IgA in the skin has ever shown evidence of spontaneous remission, and the duration of their skin disorder varies from 2 to 50 years.

Of the thirty-five patients with IgA in the skin, twenty-nine had low serum folate levels and eleven low red cell folate levels. Of the seven patients who did not have IgA in the skin, none had low red cell folate levels, but three had low serum levels. All these three patients were on dapsone at the time of study and this may have been responsible for this finding. A low red cell folate in association with an enteropathy and IgA in the skin is supportive evidence of DH, but in general its incidence is too low to be recommended as a useful screening test.

DISCUSSION

The results show that at the present time the most reliable criterion for the diagnosis of DH is the presence of IgA in the uninvolved skin. The finding is compatible with the concept that DH is a specific immunological disorder, although the exact significance of these IgA deposits is at present unknown.

In this particular series, IgA was found in thirty-five of our forty-two patients. Of these thirty-five patients, further substantiation of the diagnosis of DH was provided by the finding of a small intestinal abnormality in thirty-four. It is now well established that patients with DH have a gluten-sensitive enteropathy (Fry *et al.* 1972). In the one patient showing no evidence of enteropathy, the possibility exists of a further abnormality of the immune system; that associated with a tendency to develop malignancy. Of the seven patients who did not have IgA in the skin, only one showed a slight abnormality of the small intestine and this patient was subsequently shown conclusively to have pemphigus and not DH. There appears, therefore, to be a good correlation between IgA in the skin and the presence of a gluten-sensitive enteropathy.

In the previous reports of IgA in the uninvolved skin in DH, Van der Meer (1969) found it in only ten of twelve patients studied, but Chorzelski *et al.* (1971) reported its presence in all nineteen patients with 'typical' DH, and in none of their eight patients with 'atypical' DH. However, these latter authors did not specify their criteria for judging a DH patient as being 'typical' or 'atypical'. In the twelve patients with DH reported by Van der Meer (1969), only five were considered to have typical DH by the author and in all five IgA was found in the skin. The remaining seven patients were classified as 'atypical' DH. IgA deposits were found in five of these seven patients. It is possible that the two patients in whom IgA was not found did not have DH. Unfortunately, no other collaborative evidence, such as small intestinal findings and folate status, were discussed to clarify this point.

The presence of ARA has been reported in the sera of patients with gluten-sensitive enteropathy—both in DH and in childhood and adult coeliac disease (Seah *et al.* 1971a, 1971b). In the present study ARA was found in the sera of eight of the forty-two patients. All these eight patients had IgA in the skin. The presence of ARA is further evidence for the diagnosis of DH, but as the incidence of the antibody in the serum is relatively low, its absence does not exclude the diagnosis. The low incidence of ARA in this series may be due to the fact that many of the patients were on a gluten-free diet; it is known that the antibody disappears from the serum following treatment with a GFD and that the incidence is always higher in patients with DH or coeliac disease when they are on a normal diet (Seah *et al.* 1973a).

It is evident from this study that not many patients with DH present with a characteristic clinical pattern. In this series, only fifteen of the forty-five patients had a diagnostic clinical presentation. All these fifteen did in fact have IgA in their uninvolved skin and evidence of enteropathy. However, of the other twenty-seven patients with a less typical clinical presentation in whom the diagnosis of DH was eventually considered on clinical grounds, there was no difference in the seven without IgA and the twenty with IgA in the uninvolved skin.

In this study, histological examination of involved skin did not appear to be particularly helpful in establishing the diagnosis. The features usually considered to be characteristic of DH, i.e. subepidermal blisters, infiltration with eosinophils and papillary micro-abscesses, were present in only five of the thirty patients who had biopsies. All these five patients did in fact have IgA in the uninvolved skin and an enteropathy. Of the other twenty-five biopsies, twenty-three showed only one or two of the above histological features. All seven patients without IgA in the skin had skin biopsies taken, and the incidence of features suggesting DH was the same as in the eighteen patients with IgA deposits in their skin. In addition, it must be pointed out that the features which are characteristic of DH, namely subepidermal blisters, eosinophils and papillary micro-abscesses, are not diagnostic of DH. Following the observations of Pierard & Whimster (1961) it had been thought that papillary micro-abscesses were diagnostic, but they have since been found in other conditions, including pemphigoid (Connor, Marks & Wilson Jones, 1972).

Following the reports of Cornbleet (1951), Calnan (1954) and Alexander (1955) advocating the efficacy of sulphones, particularly dapsone, in the treatment of DH, therapeutic trials of these drugs have come to be used as a means of establishing the diagnosis of DH. In fact, this is probably the most widely used and accepted criterion for making the diagnosis at the present time. In this series, whilst all thirty-five patients with IgA deposits in their uninvolved skin had their rash controlled with dapsone, so did six of the seven patients without IgA. There are now several reports of skin diseases other than DH responding to dapsone. These include amongst others, vasculitis (Wells, 1969; Thompson *et al.*, 1973), erythema elevatum diutinum (Johnson, 1964; Vollum, 1968; Cream, Levene & Calnan, 1971), pemphigus (Floden & Gentele, 1955; Winkelman & Roth, 1960; Seah *et al.*, 1973b), papular urticaria (Hewitt, Walton & Waterhouse, 1971), and subcorneal pustular dermatosis (Sneddon & Wilkinson, 1956). The mechanism of action of dapsone in DH is unknown, and although it certainly does control the rash, the finding that it suppresses or improves other dermatoses indicates that therapeutic trials in DH are not specific in establishing the diagnosis. It is interesting to note that one patient whose rash was controlled by dapsone or sulphapyridine for 11 years was eventually shown to have pemphigus (Seah *et al.*, 1973b). In addition, three of the remaining six patients without IgA in the uninvolved skin, whose rashes were initially dapsone responsive, now no longer require dapsone and are free of their eruptions. In contrast, none of the thirty-five patients with IgA in their skin have ever shown such spontaneous remissions.

A final appraisal of the diagnostic criteria evaluated here indicates that of the forty-two patients studied, seven are considered by us not to have DH. None of these seven patients had IgA in their uninvolved skin. This is in contrast to the remaining thirty-five, all of whom had IgA deposits. Of these thirty-five patients, thirty-four had evidence of a small bowel enteropathy. It would appear that at the present time, the presence of IgA in the uninvolved skin is the simplest and most reliable way of establishing the diagnosis of DH. We believe that for a GFD to be effective in DH, it must be strict, and at the present time, life-long. Therefore, before such a diet is prescribed, the diagnosis of DH should be established by the finding of IgA in the uninvolved skin and an invariably increased lymphocytic infiltration in the small intestinal epithelium indicating a gluten-sensitive enteropathy.

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Regression of plane warts following spontaneous inflammation

AN HISTOPATHOLOGICAL STUDY

HACHIRO TAGAMI, ATSUHIKO OGINO, MASAHIRO TAKIGAWA,
SADAO IMAMURA AND SHIGEO OFUJI

Department of Dermatology, Kyoto University, School of Medicine, Kyoto, Japan

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SUMMARY

This paper describes the clinical and histopathological features in ten cases of spontaneously involuting plane warts. In all, rapid regression occurred after the sudden development of an inflammatory reaction. At an early stage a degenerative change appears in the upper epidermis and the typical features of the warts are masked. At the height of the reaction an intense mononuclear cell infiltrate in the dermis associated with epidermal spongiosis, exocytosis, cell necrosis and focal parakeratosis is found. It is suggested that the development of cell mediated immunity may be responsible for spontaneous involution of warts.

It is not uncommon for warts to disappear spontaneously; this is especially likely in plane warts in children (Naseman, 1961). Not infrequently, disappearance is preceded by an inflammatory reaction, the development of which has been shown to be a good prognostic sign (Allington, 1952). Its severity is variable; usually it is mild and it is overlooked by patients. In a severe case even small warts, of which the patient may not have been aware, become erythematous, swollen and pruritic. Patients may be alarmed by what they take to be a sudden increase in the number of lesions. We have had the opportunity of observing ten patients with inflamed plane warts. It is logical to believe that a study of flat warts with spontaneous inflammation may provide some clue to the elucidation of the mechanism which underlies the phenomenon of spontaneous involution of warts. The literature dealing with the histopathology of these involuting warts is scanty and the textbooks of histopathology contain no mention of them. Samek made histological observations on involuting warts in which he believed inflammation was induced by mental suggestion (Samek, 1931). The following report of ten cases represents the first in which systematic histopathological study has been carried out.

MATERIAL AND METHODS

Ten patients with spontaneously inflamed plane warts were studied. The diagnosis was made on the clinical appearance. Biopsy was performed on the initial visit except for one case in which this was done at the height of the inflammatory reaction, i.e. of a wart with a vesicular change. Histological sections were prepared in the usual way and stained with haematoxylin and eosin.

CLINICAL FEATURES

The main clinical data are summarized in Table 1. Five of the ten patients were male. All were under 40 years of age. Plane warts had been present for at least 3 months before the sudden onset of inflammation. The affected sites were face, neck and the dorsa of the hands, and in one case the trunk also was involved. No patient had applied topical therapeutic agents before the onset of the inflammatory signs. Usually the inflammatory reaction occurred suddenly in all the warts; in only two did it develop gradually. There was pruritus of a variable degree. Redness, swelling and slight scaling were evidence of inflammation, only rarely was vesiculation or crusting noted. The inflammatory reaction lasted a few weeks and was accompanied by complete regression of the warts.

TABLE 1. Clinical data on ten cases with inflamed plane warts

Age: 10–35 years (mean 25·7)
Male/female: 5/5
Duration of presence of warts: 8 months–several years
Distribution of warts: face 8, upper limbs 2, dorsa of hands 2, neck 2, trunk 1
Onset of spontaneous inflammation: sudden 8, gradual 2
Severity of pruritus: none 1, moderate 3, severe 6
Signs of inflammation: erythema 10, swelling 5, crusting 2, vesiculation 1
Time required for resolution of warts:

2 weeks	1
3 weeks	4
4 weeks	3
5 weeks	2
(mean 3·6 weeks)	

TABLE 2. Histopathological data from ten cases

Epidermal changes
- (i) Upper epidermis
  - Premature keratinization-like degeneration: 3
  - Parakeratosis: 7
- (ii) Lower epidermis
  - Spongiosis and exocytosis: moderate 3, marked 7
  - Individual cell necrosis: absent 7, few 1, scattered 2
- (iii) Appendages
  - Spongiosis and exocytosis around acrosyringium: 2
  - Spongiosis and exocytosis in the follicular epithelium: 1

Dermal changes
- Perivascular oedema and mononuclear cell infiltrate: moderate 6, marked 4
- Vascular dilatation in upper dermis: 4
- Extravasation of red blood cells: 2

HISTOPATHOLOGY

The histological data of the ten cases are summarized in Table 2. The feature common to all the specimens was the presence of a dense mononuclear cell infiltrate in the dermis, associated with a variable degree of epidermal change.

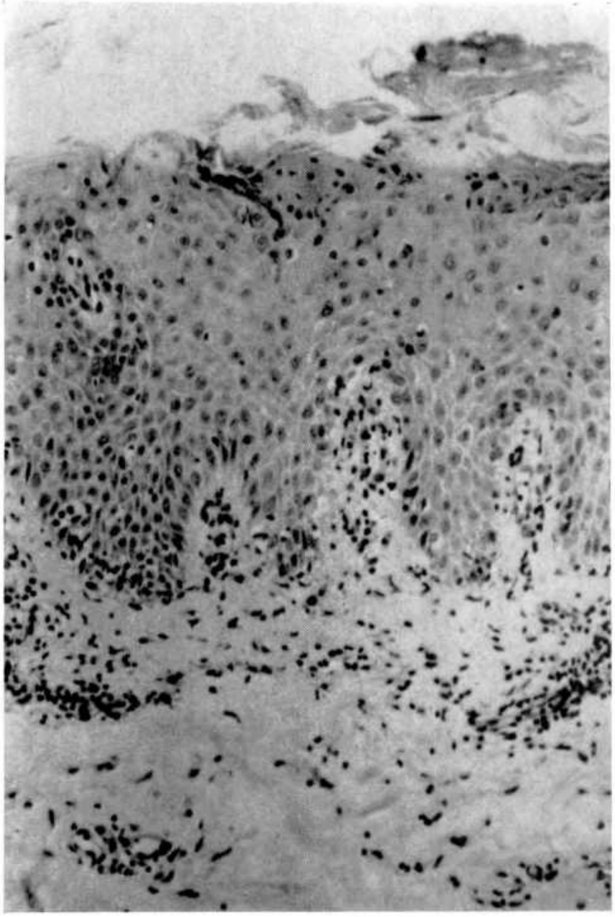

FIGURE 1. The specimen, reflecting an early phase of inflammation, shows a focal degenerative change in the upper epidermis, spongiosis and exocytosis in the basal layer, and a moderate mononuclear cell infiltrate in the upper dermis (H & E, × 100).

Epidermis

Typical viral wart changes such as a basket-weave appearance of the stratum corneum and vacuolation of the epidermal cells were rarely demonstrable. Only in those specimens with early inflammatory changes was the acanthotic epidermal architecture of a plane wart preserved. However, important features were focal spongiosis of the basal cell layer, exocytosis of mononuclear cells, and in the upper epidermis early degenerative changes resembling premature keratinization with homogenization and eosinophilic staining of the cytoplasm and nuclear pyknosis (Figs. 1 and 2). At the stage of intense inflammation, the lower half of the epidermis showed prominent spongiosis and marked cellular invasion from the dermis associated with small spongiotic vesicles, scattered individual cell necrosis and focal parakeratosis (Fig. 3). The change was variable in extent, ranging from focal spongiosis to marked necrosis (Fig. 4). Occasionally the follicular infundibulum or peri-sweat-ductal portion was involved similarly (Fig. 5).

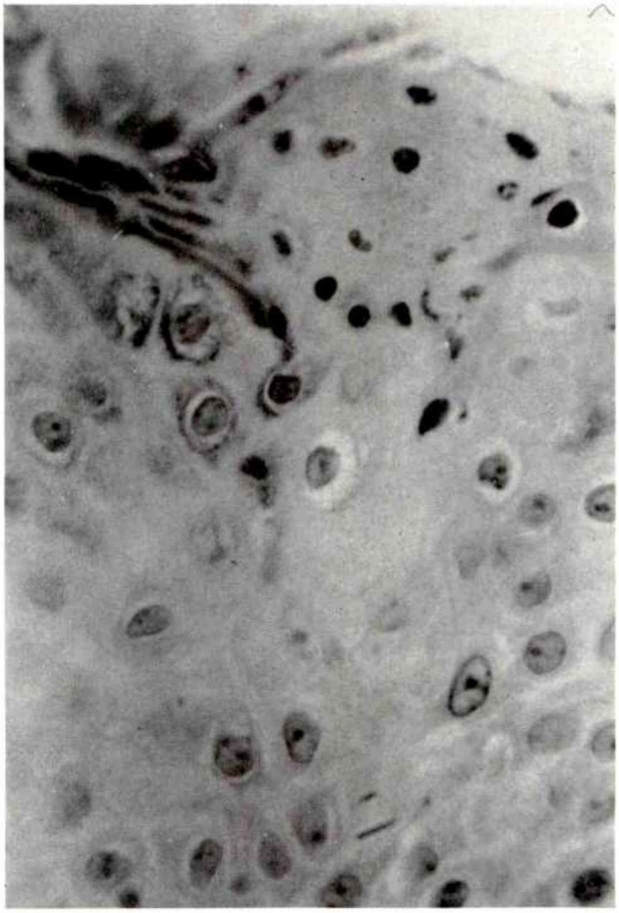

FIGURE 2. A high-power view of the upper epidermis of the same specimen. A focal wedge-shaped degenerative change resembling premature keratinization is noted at the centre, which consists of homogenization and eosinophilic staining of the cytoplasm and pyknotic nuclei. Slightly vacuolated cells are still present in the surrounding stratum granulosum (H & E, × 400).

Dermis

Perivascular oedema and a dense mononuclear cell infiltration were the prominent changes especially in the upper dermis (Fig. 6). Polymorphonuclear leukocytes were not observed. The small blood vessels in the upper dermis were dilated in places. In a few specimens haemorrhage was noted, and red blood cells as well as mononuclear cells penetrated into the overlying spongiotic epidermis.

DISCUSSION

This study has revealed that, during involution, the histopathological picture of plane warts was

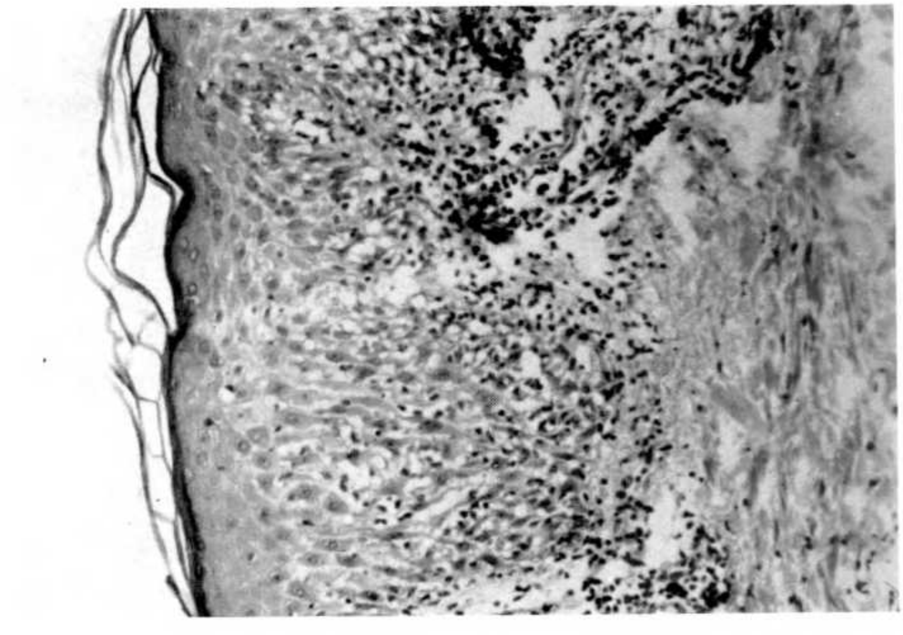

FIGURE 3. Markedly spongiotic lower epidermis is invaded by mononuclear cells which infiltrate densely in the upper dermis (H & E, × 100).

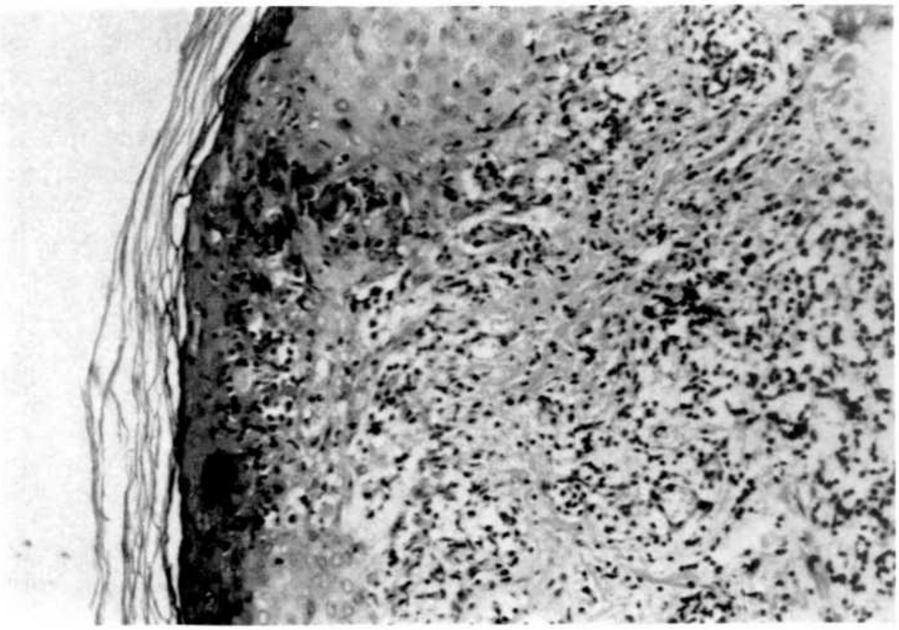

FIGURE 4. There is massive necrosis in the upper epidermis. The lower half of the epidermis shows prominent oedema and exocytosis of mononuclear cells (H & E, × 100).

greatly altered or even effaced by marked inflammatory changes. There was a prominent inflammatory reaction in the dermis composed of a dense mononuclear cell infiltrate and degenerative alterations in the epidermis. Superficially one could consider such diagnoses as subacute dermatitis, pityriasis lichenoides, chronic pigmented purpura, erythema multiforme, lichen striatus, lichen planus, hand, foot and mouth disease, or fixed drug eruption. The histological changes noted in our cases appear to correspond to those reported by Samek (1931) in involuting warts following suggestion; he observed disappearance of the epidermal changes characteristic of plane warts, with a dense cellular infiltration in the dermis in addition to exocytosis into the epidermis, oedema and hyperaemia. Ikegami (1960) performed histological studies on involuting plane warts after repeated intralesional injections of purified vaccine lymph (Yaoi antigen) and noted similar changes. Minami & Minami (1959) carried out a detailed histological study on ordinary plane warts and found the typical vacuolated cells in only thirty-three of fifty-five cases. According to these authors, healing was inversely correlated with the number of vacuolated cells present. Cures were observed in twelve of fifteen cases with inflammatory changes in the dermis, eight of whom showed similar histological changes to those found in our cases.

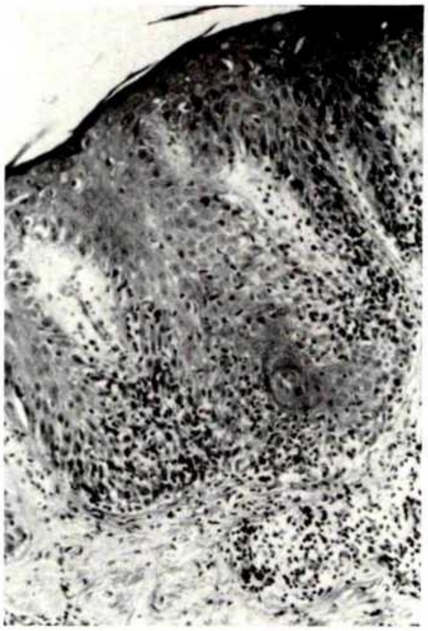

FIGURE 5. Spongiosis and dense exocytosis of mononuclear cells are also noted in the peri-sweat-ductal portion of the epidermis (H & E, × 100).

Although the histological features differed from those of ordinary plane warts, the clinical appearance left no doubt about the accuracy of the diagnosis. Most cases showed involution of the warts within one month of the onset of inflammation.

The mechanism involved in the phenomenon of spontaneous resolution of warts are still unknown, but an immunological response has often been suspected. In regard to circulating antibodies, Ogilvie (1970) demonstrated a relation between the appearance of antiviral antibodies and the regression of warts; those whose warts underwent spontaneous resolution had also developed antibodies. However, Ogilvie speculated that the resolution of warts depended on a cell mediated immune mechanism and that the antibody response occurred during the destruction of the infected cells when the relatively inaccessible virion antigens were exposed to the antigen recognition system. A high incidence of IgG antibody was reported in sera from patients whose warts were successfully treated by topical medicaments (Bunney *et al.*, 1971). Pyrhönen & Penttinen (1972), however, pointed out that, even though healing showed some correlation with the presence of IgG antibodies, the incidence of these antibodies was not particularly high in patients whose warts had regressed. Meanwhile, Allison (1966) demonstrated, in work concerning the humoral and cellular immune responses in the regression of viral papillomas in rabbits, that circulating antibody was not responsible for the resolution of the lesions, and that the main factor involved was the cell mediated immune response. The inflammatory changes observed by us in spontaneously involuting plane warts, i.e. intense mononuclear cell infiltration in the dermis, extending into a damaged overlying epidermis and the absence of polymorphonuclear leukocytes, could indicate the importance of the role of a cell mediated immune response. Studies on molluscum contagiosum, another viral epidermal tumour, offer a pertinent analogy (Pinkus & Frisch, 1949; Goldschmidt & Kligman, 1958); persons presumed to have been sensitized through previous

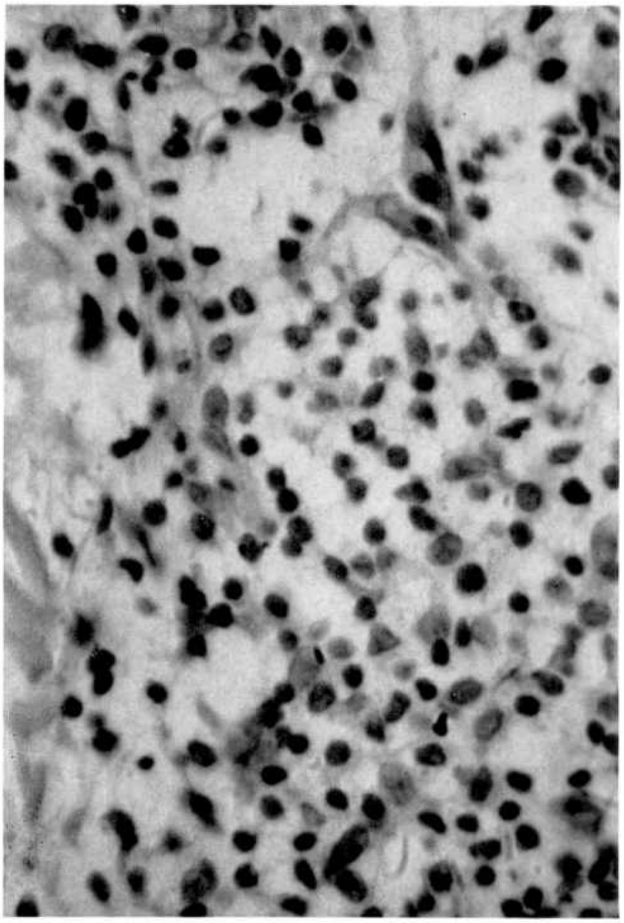

FIGURE 6. A high power view of the mononuclear cell infiltrate. There is marked perivascular oedema (H & E, × 400).

infection were not only immune to attempts at experimental infection but also revealed delayed-type inflammatory reactions which were characterized histologically by a perivascular lympho-reticular cell response. So far, however, no conclusive experimental studies using skin tests in patients with spontaneously regressing warts have been performed. The general conclusion drawn from these data is that if an immunological response is involved in the spontaneous resolution of warts it is likely to be cell mediated in type.

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Mycetoma formation in *Trichophyton rubrum* infection

CARROLL F.BURGOON Jr, F.BLANK, WAINE C.JOHNSON AND
SARAH F.GRAPPEL

Skin and Cancer Hospital of Philadelphia, Department of Dermatology, Temple University Health Sciences
Center, 3322 North Broad Street, Philadelphia, Pennsylvania 19140, U.S.A.

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SUMMARY

An adult male patient with a chronic *Trichophyton rubrum* infection of the feet, toe nails and groin, for 15 years, developed on the dorsum of the right foot a tumour with draining sinuses. Histological examination of tissue from the growth revealed granulomatous inflammation with abscesses containing granules characteristic of mycetoma. *T. rubrum* was cultured from skin scrapings and toe nails. The concurrent complete clearing of the superficial lesions and the mycetoma during treatment with griseofulvin, as well as the disappearance of complement fixing antibodies against *T. rubrum* antigen, indicate that this hitherto unreported complication of a dermatophyte infection may be related and may not be coincidental to the infection.

We report a patient who had had persistent *Trichophyton rubrum* infection of the skin of the feet, groin, and the nails of the toes for over 15 years and who subsequently developed a mycetoma on the dorsum of one foot. We consider this event a hitherto unreported complication of a dermatophyte infection of the skin.

Mycetomas are usually considered to be reactive inflammatory nodules of the skin and subcutaneous tissue with sinus tracts exuding material, usually containing granules made up of colonies of actinomycetes or true fungi. The subcutaneous inflammatory disease involving the skin of our patient fulfils the clinical and histological criteria of mycetoma, although no granules or organisms were found on direct microscopic examination of exudate from the sinus tracts. For a discussion of organisms causing mycetoma, the reader is referred to the paper by Macotela-Ruiz presented at the recent International Symposium on Mycoses (Macotela-Ruiz, 1970) and to the monograph by Mahgoub & Murray (1973).

MATERIALS AND METHODS

Smears

Exudate was taken from sinus tracts and smeared on clean dry slides for routine direct microscopic examination in 10% potassium hydroxide solution and after staining with Gram's and Wright's stains.

Cultures

Exudate from sinus tracts was inoculated on Sabouraud's and Littman's oxgall agar, and incubated at

20°C. Cultures for fungi were not made from the surgical specimens. Discharge from sinus tracts was collected for aerobic and anaerobic bacterial culture.

Histopathology

All biopsy specimens were prepared routinely, using 10% buffered formalin fixative solution. The specimens were embedded in paraffin blocks and the sections were stained with haematoxylin and eosin. The sections were also studied using special staining techniques, including periodic acid–Schiff (PAS), PAS with diastase digestion, Gram, and Gomori methenamine–silver nitrate stains (Luna, 1968).

Serology

Test antigens used were a saline extract of mycelia and cell wall polysaccharides of *T. rubrum*. The patient's serum was analysed for the presence of circulating antibodies by charcoal agglutination, complement fixation, and immunodiffusion techniques (Grappel, Blank & Bishop, 1971).

CASE REPORT

The patient was a 52-year-old Caucasoid male, who complained of a slowly enlarging non-tender growth which appeared on the dorsum of the right foot during the summer of 1963. The surface of the tumour intermittently became eroded and exuded purulent material from the resulting sinus tracts.

The past medical history was noteworthy, for he had had for 15 years non-seasonal asthma of sufficient severity to require oral corticosteroid (prednisone 20 mg daily in divided doses) for control of symptoms for the last 6 years. He was known to be allergic to penicillin.

First hospital admission

He was admitted on 31 August 1964 to a surgical service. On physical examination, a soft non-tender mobile tumour measuring 3 × 4 cm with several small draining sinuses was found on the dorsum of the right foot overlying the distal second to fourth metatarsals. A culture of the exudate from the sinus tracts for bacteria grew a mixed flora (*Staphylococcus albus*, coliforms, haemolytic streptococci). Routine studies, including urinalysis, complete blood count, and serum cholesterol were within normal limits. Radiological examination of the chest showed evidence of emphysema. X-ray examination of the right foot showed a soft tissue tumour overlying the second to fourth metatarso-phalangeal joint spaces but no evidence of bony abnormality; there was medial wall calcification of the anterior and posterior tibial arteries and their respective branches within the foot.

The mass was dissected free, down to the level of the deep fascia, and the skin flaps closed over the deject. The operative site healed satisfactorily. The gross specimen consisted of greyish-pink tissue measuring 3·5 × 6·5 cm. Microscopic examination of sections from the specimen was reported by the surgical pathologist to show 'nodular collections of lipid laden macrophages surrounded by an inflammatory infiltrate in the dermis'. These findings were interpreted as 'consistent with xanthoma with secondary infection'.

Second hospital admission

The growth recurred and he was re-admitted on 7 September 1965 and was seen in dermatological consultation. He stated that for 15 years he had had dryness and scaling on the plantar surfaces of the feet, thickening of the toe nails, and a recurrent, pruritic rash in the groins. About 8 months after the excision of the growth on his right foot, several small 'lumps' appeared parallel to the scar of the excision site and slowly enlarged. Small erosions appeared on the surface of the nodules and discharged 'bits of yellowish-white' material (Fig. 1a). The corticosteroid dosage had been continued (prednisone 20 mg/day in divided doses) since the first admission.

Physical examination. There was an erythematous scaling eruption with sharply defined borders located on the proximal medial aspects of the thighs, the pubic area, and the buttocks. The toe nails were discoloured and subungual keratotic debris was found beneath the nail plates. The soles showed diffuse scaling with slight erythema. There was a linear scar involving the skin overlying the distal portions of the metatarsals of the right foot. Proximal to the scar and surrounding it, the skin was dusky and oedematous, and felt infiltrated on palpation. Removal of crusts from several inflammatory areas revealed draining sinuses. The femoral and inguinal lymph nodes were significantly enlarged.

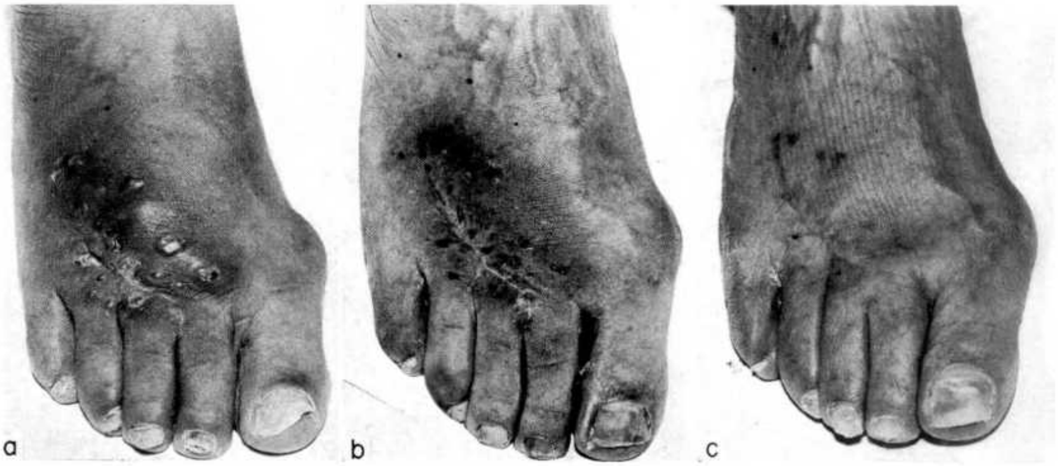


FIGURE 1. (a) Recurrent tumour showing dusky coloured infiltrated growth with multiple crusted and draining sinuses surrounding scar of original excision; note onychomycosis. (b) Mycetoma has cleared after 13 months of treatment with griseofulvin. Scarring post-inflammatory vascular changes and regression of nail infection. (c) Appearance of foot 5 years after treatment showing scarring at original site and recurrence of nail infection.

RESULTS

Direct examination and cultures

Septate hyphae were found on direct microscopic examination of scrapings from the skin of the groin, buttocks, plantar surfaces of the feet, and from beneath the toe nails. *T. rubrum* was grown from each of these locations. Gram-stained smears of the exudate from the sinus tracts showed epithelial cells, polymorphonuclear leukocytes, and a number of multinucleated giant cells, but no granules were found. No pathogenic organisms were grown on culture of the exudate from the tumour on either Sabouraud's or Littman's oxgall media, nor were pathogenic bacteria grown on bacterial cultures.

Histopathology. The original sections of the tumour surgically excised from the right foot during the first period of hospitalization were re-studied. The haematoxylin and eosin stained sections showed multiple nodular cellular infiltrates composed of histiocytes with foamy, granular cytoplasm, macrophages, fibrocytes, plasma cells and lymphocytes. The centre of many of the nodules consisted of an abscess composed mainly of polymorphonuclear leukocytes; many of these abscesses contained one

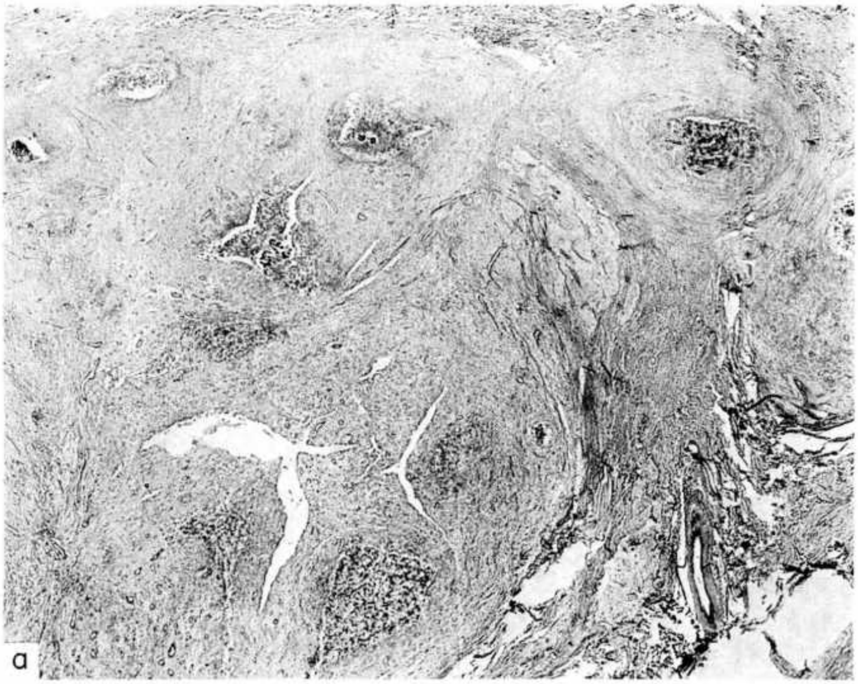
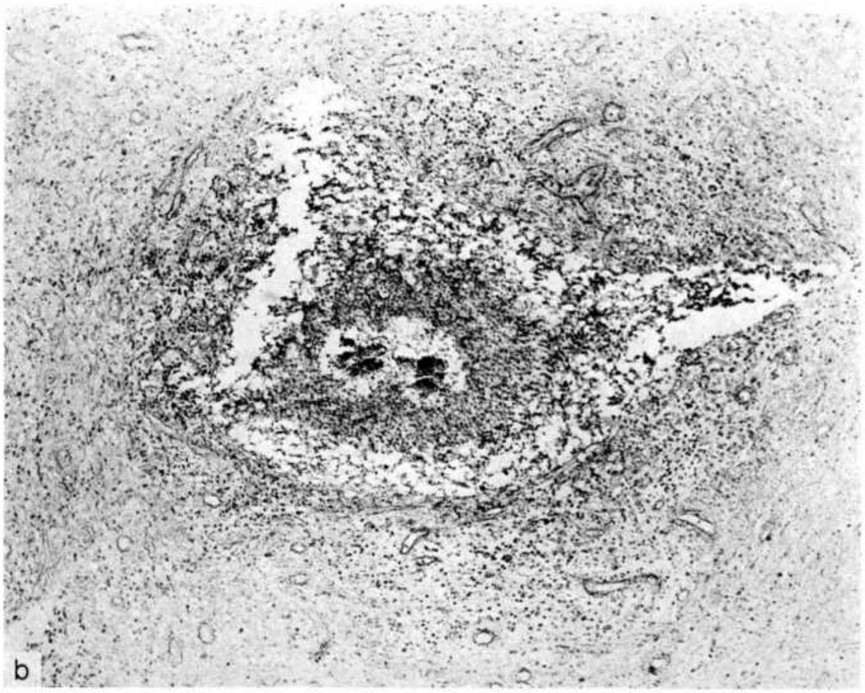


FIGURE 2. (a) Abscess formation in nodular cellular infiltrate in dermis showing granules (PAS, × 50).



FIGUER 2. (b) Higher magnification showing granule formation in centre of abscess (PAS, × 175).

or more granules. The centre of the granules appeared amorphous or granular while their periphery consisted of elongate, club-like structures (Fig. 2). The stroma within and about the nodules was oedematous and showed fibrosis and proliferation of capillaries. The centre of the granules was PAS reactive, and the club-like structures stained yellow with the counterstain in sections treated with PAS after digestion with diastase and counterstained with picric acid. There was a linear arrangement of PAS reactive material within this central amorphous and granular material, which resembled fungal filaments, but these were not distinct and could have represented an artifact. The central and peripheral areas of many of the granules were reactive with the Gram stain and resembled filaments. Some of the areas within the granules stained with the Gomori methenamine–silver technique but no organisms could be recognized. The histological sections were reviewed by consultants who concluded that there was a mycetoma in the histological sense of the definition, that the visible 'grains' were formed by a fuchsinophilic substance, and that no fungal or actinomycotic, bacterial elements were detectable in the material (Ajello, 1969; Destombes, 1969).

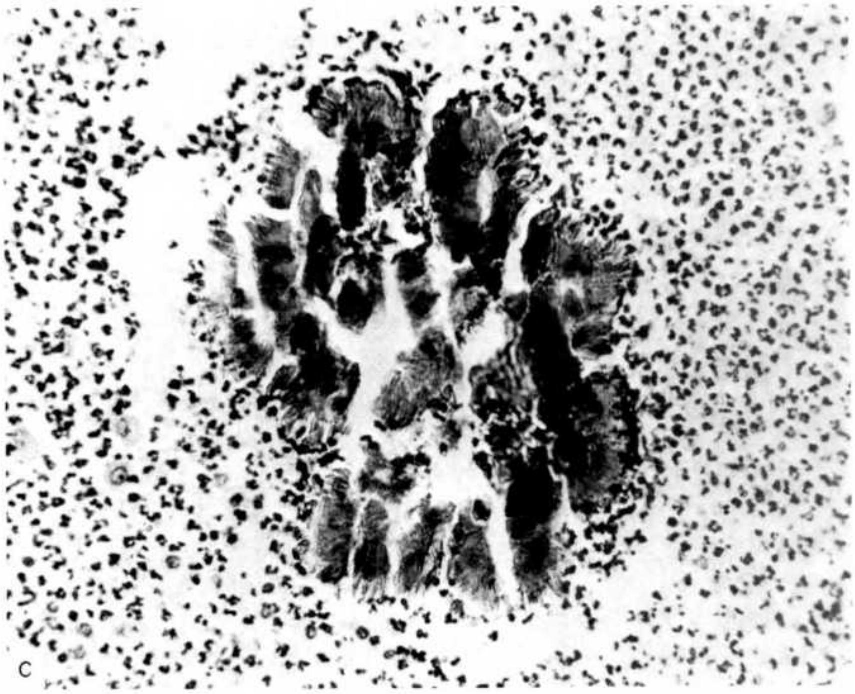


FIGURE 2. (c) Granule in centre of abscess in (b) showing amorphous fuchsinophilic centre with elongated club-like structures at periphery of granules (PAS, × 430).

Two additional 4 mm punch biopsy specimens were obtained during the second hospital admission, but these did not include the deep dermis and subcutaneous tissue. The sections showed only granulomatous inflammation. No abscesses or grains were seen in these specimens.

Serological investigations. Samples of blood were drawn on 9 September 1965, 14 November 1968, and 23 February 1971. The serum was analysed for the presence of circulating antibodies by charcoal agglutination, complement fixation, and immunodiffusion. The saline extract of mycelia and polysaccharides of *T. rubrum* were used as antigens. Significant serological reactivity, however, was obtained only with the saline extract of mycelia. The results of these investigations are presented in Table 1.

Treatment and clinical course. The prednisone dosage was continued (20 mg/day) throughout the period of treatment because of the severity of the asthma. The patient was given 1 g of griseofulvin by mouth each day after the evening meal. Within 2 weeks clinical signs of infection on the buttocks and groin had disappeared completely. After 4 weeks of treatment, the scaling on the soles had diminished approximately 85%, while the tumefaction of the dorsum of the foot had regressed approximately 50%. The infection of the toe nails remained unchanged. By 12 weeks, the scaling on the soles had cleared completely, but there had been no further improvement in the inflammatory area on the dorsum of the foot. The dosage of griseofulvin was increased to 2 g by mouth after the evening meal. After 8 weeks of treatment with this dose, the tumefaction diminished by approximately 75–85%; by 12 weeks, the tumefaction had regressed completely, except for scarring and a post-inflammatory vascular reaction observed on change of temperature or on placing the foot in the dependent position. Treatment with griseofulvin was continued at a dosage of 1 g/day for an additional 8 months, then stopped (Fig. 1b). Throughout the course of treatment, the infection of the toe nails slowly improved but did not resolve completely. He has been seen at regular intervals since treatment was discontinued and there has been no recurrence of the mycetoma, although the nail infection has relapsed (Fig. 1c).

TABLE 1. Serological tests

Date of test	Saline extracted antigen		
	Immunodiffusion	Complement fixation	Charcoal agglutination
September 1965	–	+	+*
November 1968	+†	–	+*
February 1971	+‡	–	–

* Titre 1:16 (0·05 ml serum); † two precipitin bands; ‡ one faint precipitin band (serum concentrated × 5).

DISCUSSION

The diversity of agents, ranging from actinomycetes to moulds, which are capable of producing the same clinical picture, is one of the peculiarities of mycetoma. Rarely, the cutaneous lesions caused by a staphylococcal infection (botryomycosis) may superficially resemble mycetoma clinically and histologically, but Gram-stained sections show the club-like projections of the granules to be made of masses of staphylococci (Waisman, 1962).

In experimentally produced mycetomas in animals, it has been shown that the development of maduromycotic and actinomycotic grains is a function of time. In the dynamic formation of grains, hyphae are abundant in young grains but sparse or absent in old ones. This observation in part accounts for the difficulty in making a diagnosis of the pathogen from histological preparations of tissue specimens (Avram, 1967). Until recently, identification of the causative organism in mycetomatous infections depended on histological and cultural methods. The diagnosis is sometimes difficult because histological identification of the organism is imprecise and because of laboratory contaminants of cultures from exudates. A demonstration of precipitating and complement-fixing antibodies in sera of patients with mycetomas has been useful in diagnosis or in the confirmation of diagnoses of mycetomas due to several species of fungi and actinomycetes (Murray & Mahgoub, 1967; Avram & Nicolau, 1969).

Serological studies in our laboratory have shown that, in an analysis of sera from patients with *T. rubrum* infections, 48/150 (32%) reacted with mycelial extracts of *T. rubrum* by charcoal agglutination tests with titres up to 1:64; whereas 3/50 (6·0%) of sera from non-infected adults reacted by this method of analysis, with titres below 1:16.

Sera from infected patients also contained precipitins (24/133; 18%) and complement-fixing antibodies (20/62; 32%), which are probably more significant since they were not found in any sera from non-infected individuals (Grappel *et al.*, 1972).

In our patient, complement-fixing antibodies were detected only in sera taken while the mycetoma was present. The absence of complement-fixing antibodies following resolution of the inflammatory process, and the disappearance of precipitating antibodies with clearing of the *T. rubrum* infection on the skin, even though the nail infection persisted, may be further evidence for relationship between the *T. rubrum* infection and the events observed. We have observed a similar precipitin, agglutination, and complement-fixing antibody decrease to a saline extract of *T. sulfureum* in a child with tinea capitis and kerion formation caused by *T. sulfureum* following griseofulvin-induced resolution of the infection (Grappel *et al.*, 1972).

T. rubrum infection of skin and nails is common, but the concurrent appearance of the mycetoma syndrome is hitherto unreported. A reasonable explanation for this unusual event may be found in the patient's long term treatment with corticosteroids. Although the dosage would not be considered sufficient to produce immunosuppression, it may have compromised the host's immuno-competency.

The question as to whether *T. rubrum* caused the mycetoma cannot be definitely answered since the organism was not demonstrated in or cultured from the mycetoma. Other micro-organisms known to cause mycetoma, *Nocardia brasiliensis* excepted (Latapi, 1960), have not usually been shown to be sensitive to griseofulvin. Therefore, regression of the mycetoma with griseofulvin treatment and the serological changes suggest a causal relationship between *T. rubrum* and the mycetoma.

Addendum

After the acceptance of our manuscript, Baylet *et al.* (1973) reported four scalp mycetomas characterized by the association of a subcutaneous lesion with white granules and isolation of *Microsporum ferrugineum* on culture.

ACKNOWLEDGMENTS

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Growth on Sabouraud's agar of dermatophytes obtained by conventional scraping and by stripping with vinyl tape

E.A.KNUDSEN

Department of Dermatology, The Finsen Institute, Copenhagen, Denmark

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SUMMARY

In a preliminary investigation it was demonstrated that dermatophytes may grow from stratum corneum strippings placed direct on Sabouraud's dextrose agar.

In the current study the results of such culture from stratum corneum strippings were compared with those of culture by the conventional technique.

Out of 930 fungal tests, growth was obtained by both methods in eighty-two cases. In thirty-one cases growth of dermatophytes was obtained solely by the conventional technique and in eight cases solely by the tape method. Six species of dermatophytes were represented in the culture experiments.

This significant difference between the number of positive cultures is due to the strip technique being in some cases less suited for collecting scales, as there are no signs that the vinyl tape inhibits fungal growth.

The strip technique is easy and practical, but as it yields less than the conventional technique, it is not suitable as a routine procedure.

The conventional technique of diagnosing fungous infections of the skin is very time-consuming.

In 1961, Keddie, Orr & Liebes described a quick and easy technique which might possibly be used as a routine method for the laboratory diagnosis of ringworm infections. These authors stripped off the stratum corneum by means of vinyl tape, which they then placed on the surface of Sabouraud's agar.

In a preliminary study (Knudsen, 1971) the present author demonstrated that dermatophytes may grow from stratum corneum strippings.

The purpose of the current study was to compare the results of conventional culture and culture from strippings of the stratum corneum.

MATERIAL AND METHOD

The analysis comprises investigations performed in the Out-patient Department during a period of 9 months.

During this period a total of 1135 investigations for fungi were carried out. In 205 of the cases the sites involved were the nails or mucous membranes, for which the strip technique is inapplicable. The remaining 930 samples provided the basis of a comparison between the results of conventional culture and culture from the stratum corneum stripping.

Technical procedure

Material for the fungous investigation was collected, when possible, both by scraping (conventional technique) and by stripping with vinyl tape.*

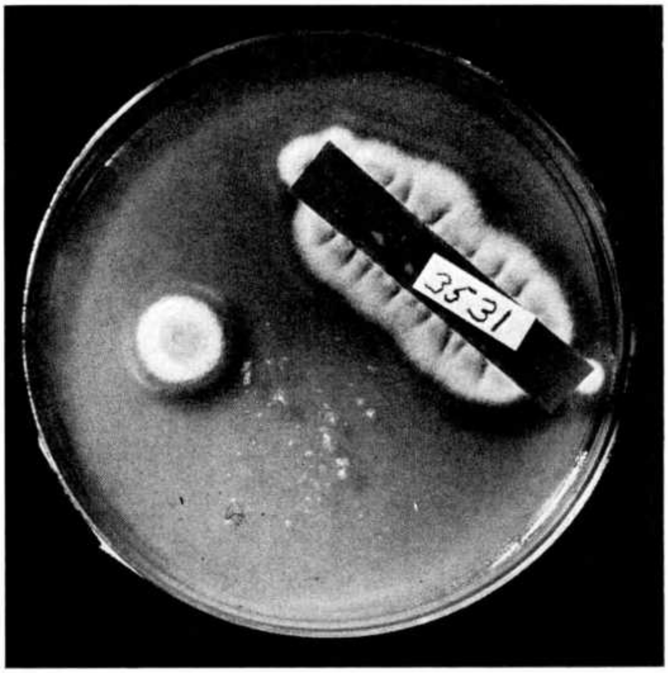

FIGURE 1. A 1-week culture of *Epidermophyton floccosum* on Sabouraud's dextrose agar.

If there was only one suitable test site of small extent, stripping with tape was done first, followed by scraping. In other cases two equally suitable test areas were used.

After the stripping the piece of tape was placed, adhesive side down, on one half of a Sabouraud's dextrose agar plate (with penicillin, streptomycin, and actidione added), and some of the scrapings were seeded on the other half of the plate.

The plate was incubated at 26° C and the two halves were examined for growth for up to 3 weeks (Fig. 1).

KOH preparations were also made, but as the results of these examinations are not relevant to the present investigation they are not included here.

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* Scotch brand tape 681, Minnesota Mining and Manufacturing Co., Los Angeles, California.

RESULTS

Number of positives by conventional culture compared with tape culture

As is evident from Table 1, growth was obtained by both methods in eighty-two out of 121 comparisons. Six dermatophyte species were represented.

TABLE 1. Comparison of the growth on Sabouraud's dextrose agar of different dermatophytes obtained by the conventional method and from skin strippings (strip no. 1)

Species of dermatophytes	No. of cases Growth by conventional culture and by tape culture	Growth by conventional culture only	Growth by tape culture only	Total no. of positive cultures
Trichophyton rubrum	37 (66%)	16 (29%)	3 (5%)	56
T. mentagrophytes	21 (62%)	11 (32%)	2 (6%)	34
T. verrucosum	1	1	0	2
Microsporum canis	5	0	1	6
M. gypseum	0	0	1	1
Epidermophyton floccosum	18 (86%)	2 (10%)	1 (5%)	21
Non-determinable	0	1	0	1
Total	82 (68%)	31 (26%)	8 (7%)	121

TABLE 2. Comparison of growth on Sabouraud's dextrose agar of dermatophytes obtained from different sites by the conventional method and by skin strippings (strip no. 1)

Type of infection	No. of cases Growth by conventional culture and by tape culture	Growth by conventional culture only	Growth by tape culture only	Total no. of cultures
Tinea pedis	41 (60%)	23 (34%)	4 (6%)	68
Tinea manum	3 (43%)	3 (43%)	1 (14%)	7
Tinea cruris	23 (89%)	3 (12%)	0	26
Tinea corporis	13 (81%)	1 (6%)	2 (13%)	16
Tinea capitis	2 (50%)	1 (25%)	1 (25%)	4
Total	82	31	8	121

In thirty-one cases growth of dermatophytes was obtained only by the conventional technique and in eight cases only with the use of tape.

This difference in the number of positive cultures by the conventional technique and by the tape technique was significant ($P = 0{\cdot}001$), but nevertheless the eight cases showing growth from tape, but not by the conventional method, were a bonus provided by the tape.

In Table 2 the results are analysed by region. It shows that the tape technique failed mainly when the fungal diseases affected the hands and feet. In tinea corporis and cruris the tape and the conventional method gave approximately equal results.

Comparison of the growth rates

During the first 5 months of the study, the plates were read both 1–2 and 3 weeks after inoculation in order to compare the growth rate (defined as the time elapsing from inoculation before signs of fungal growth appeared) in conventional culture and in tape culture. This analysis comprises fifty-two comparisons.

As is apparent from Table 3, it was not possible to demonstrate any difference in growth rate.

TABLE 3. Comparison of the growth rates for dermatophytes by conventional culture and by tape culture

No. of cases	Growth after 1 week	Growth after 2 weeks	Growth after 3 weeks	Total
Growth by conventional culture	35	15	3	52
Growth by tape culture	36	15	1	52

TABLE 4. Growth of dermatophytes in relation to depth of stripping

Strip no.	No. of cases
1+2+3	23
1+2	2
1+3	2
2+3	1
1	0
2	2
3	0
Total	33

Number of positive cultures in relation to strip depth

In 328 of the cases, stripping was done three times (in the same site), and the three pieces of tape were placed on one half of the agar plate.

Growth of dermatophytes occurred in thirty-three of these 328 cases. The distribution by strip number may be seen from Table 4.

Thus, the use of a second strip resulted in a gain of three positives, whereas the addition of a third strip did not further increase the number of positive cultures.

DISCUSSION

The result of a test for fungi depends *inter alia* upon the time and care expended on collecting the material.

In our department, the collecting has been done for many years by the technical staff, but since the laboratory also serves as a mycological diagnostic centre for many dermatological practitioners, it receives fungal material from them too.

Out of 1000 randomly selected samples from dermatological practitioners, 147 proved positive on culture, whereas growth of fungi was obtained from 207 out of 1000 samples from the department. This difference, which is significant ($P = 0{\cdot}01$) and by no means a new experience, might be due to the laboratory staff having a better collecting technique, but it might also imply that dermatological practitioners perform tests for fungi on indications different from those prevailing in the department.

Since about 75% of the samples studied by the laboratory are from dermatological practitioners, there was reason to investigate whether it was possible to recommend a technique which would give a higher yield in dermatological practice. According to our preliminary experience (Knudsen, 1971) of collection and culture by means of stratum corneum strippings, this method is well adapted to the conditions of practice.

As is evident from the current study, however, tape culture gave results somewhat inferior to the conventional collection and culture technique. There being no signs that the tape inhibits growth of fungi, the difference must be due to the strip technique being in some cases less suited for collecting scales.

In tinea corporis and cruris the tape method is as effective as the conventional technique and might be considered for routine use. On the other hand, the nature of the stratum corneum of the palm and sole makes it difficult satisfactorily to load the tape with horny substance. This latter is more easily obtained if the skin of the palm or sole is lightly scraped before the tape is applied. Where the skin is greatly macerated the tape is not sufficiently adhesive. In very hairy areas the stripping has to be preceded by careful shaving.

A practical advantage of the tape method is that the material is fixed and that a piece of tape is easy to mail, whilst it may be more difficult to handle loose scales, hair, and pieces of nail. If loose material is to be collected on a glass plate, it can as easily be collected by means of a piece of tape and mailed between two slides.

The use of vinyl tape for collecting and culturing fungi is convenient, but owing to its shortcomings we cannot accept it as a routine method in hospital practice.

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p-Cresol: cause of ink-induced hair depigmentation in mice

WALTER B. SHELLEY

Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

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SUMMARY

p-Cresol has been identified as the active chemical in a laundry-ink responsible for the previously reported contact depigmentation of hair in agouti CBA/J mice. *p*-Cresol has been shown to induce depigmentation of both skin and hair in agouti as well as black mice. In the agouti mice there were bands and whorls, as well as plaques of white hair. However, the most striking finding was an occult hair depigmentation wherein the new hair shafts were white except for the tips. Such pigment-loss was not apparent on surface examination of the fur, becoming evident only on parting the hairs. All of these patterned hair pigment losses have been related to a unique sensitivity of the follicular melanocytes to the toxic effect of *p*-cresol during the earliest days of new hair formation, that is, during the initial anagen phase.

There is a growing awareness of the occupationally acquired leukodermas which result from contact exposure of individuals to germicides, anti-oxidants and other materials containing phenolic derivatives (Ito, Nishitani & Hara, 1968; Kahn, 1970; Gellin, Possick & Davis, 1970; Malten *et al.*, 1971; Telegina & Boiko, 1972). With reference to this, our serendipitous discovery (Shelley & Raque, 1972) that contact with laundry-ink could induce depigmentation in mice seemed of especial interest. The immediate question was what component of the ink was responsible for the pigment loss. The present report describes the experiments undertaken to answer this question.

MATERIALS AND METHODS

One hundred female CBA/J agouti mice were obtained from Jackson Laboratories, Bar Harbour, Maine. The strain colour gene was the wild type allele + or A agouti. Hair pigmentation was studied following plucking or clipping the caudal half of the back, and repeated application of Aero® brand indelible laundry-ink (Aero Ink Company, New York City) or its pure component chemicals, each in a concentration of 0·5% in acetone. All chemicals were applied topically three times weekly for 6 weeks to the epilated or clipped area. Application was achieved by a mist spray from a tuberculin-syringe-30 needle unit to the point of minimal wetting of the skin surface. Observations of hair colour were made weekly for the subsequent 6 months. Comparable observations were made on control mice and on mice painted with 8-hydroxyquinoline (Searle, 1972). The effects of *p*-cresol (0·5% in acetone) were investigated in an additional thirty black, 6-week-old male mice of the C57BL/6J strain.

Microscopic observations were made on plucked sample post-treatment hairs as well as on H & E and dopa-stained skin biopsies of areas of the non-pigmented and of the normally pigmented hair.

RESULTS

The topical application of *p*-cresol reproduced the patterned white hair seen following the application of the laundry-ink (Shelley & Raque, 1972). In two of the five agouti mice treated, target rings were seen (Fig. 1c and d). This was also seen in two of five mice which had been clipped. In two others

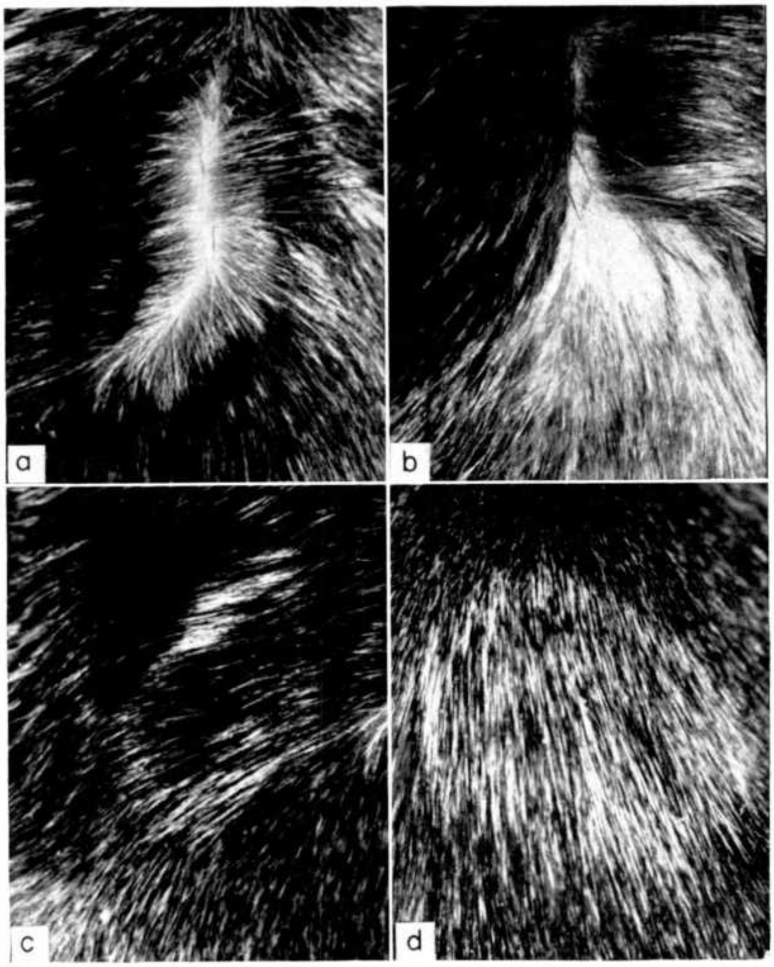

FIGURE 1. Depigmentation of new hairs of the back of female CBA/J agouti mice. Loss of ability to pigment new hairs followed plucking and painting area with 0·5% *p*-cresol in acetone three times a week for 3 weeks. (a) New hairs showing white shaft but with dark melanized tips. Such pigment absence evident only on parting the hair. (b) New white hairs appearing in triangular plucked area. (c) Patterned depigmentation duplicating findings after application of laundry-ink. (d) Patterned pigment loss grossly visible.

there was regrowth of totally white hair in the plucked area to which *p*-cresol solution had been applied three times weekly for 4 weeks (Fig. 1b). However, the most consistent and striking finding in these mice treated with the *p*-cresol was the occult loss of pigment in the hair. By occult we refer to the fact that the surface colour gave no clue to the underlying loss of pigment from 90% of the hair shaft. This depigmentation became apparent only after parting the hair (Fig. 1a). The extreme tip of the new hair growth was brown. Below this was a narrow black band, the remainder of the shaft being white. The dark surface colour was determined by the distal pigmented tip. The remarkable depigmentation of the major portion of the shaft was successfully camouflaged until the hair was separated and viewed laterally. The same phenomenon was seen at 6 months. Examination of the hairs showed pigment in the tip, with the remainder of the hair shaft and root without melanin granules (Fig. 2). Dopa stains of the skin were negative for dopa-positive cells in the follicles of the areas with depigmented hair.

Ortho- and *meta*-cresol, as well as nigrosin, shellac, sulphuric acid, and acetone, produced no colour change (Table 1). However, phenol had a depigmenting effect on the shaft, which also exempted the tip. It did not produce patterned or banded surface whitening as did the ink. The colour loss was seen at 2 weeks but has largely faded by 6 months, whereas the *p*-cresol effect persisted.

8-Hydroxyquinoline regularly produced the dramatic, variable patterned, surface hair colour losses

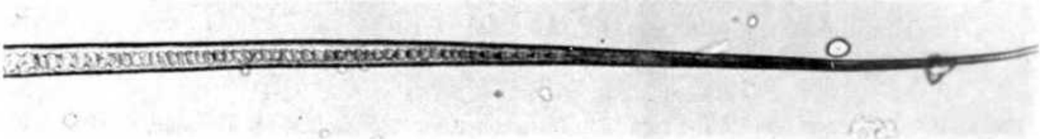

FIGURE 2. Magnified view of hairs from Fig. 1a showing non-pigmented shaft and melanized tip following *p*-cresol treatment (× 160).

TABLE 1. Results of topical application of Aero® laundry-ink and its components on pigmentation of new hair regrowth after plucking of lower back of CBA/J mice (five mice in each group)

Chemicals	2 weeks		6 months	
	White surface patterns	Dark tip,* white shaft	White surface patterns	Dark tip,* white shaft
o-Cresol	–	–	–	–
m-Cresol	–	–	–	–
p-Cresol	+	+	+	+
Laundry-ink 5%	+	–	Did not survive	
Laundry-ink 10%	+	–	Did not survive	
Laundry-ink 50%		Lethal		
Nigrosin	–	–	–	–
Phenol	–	+	–	slight
Shellac	–	–	–	–
Sulphuric acid	–	–	–	–
Acetone control	–	–	–	–
8-Hydroxyquinoline control	+	–	+	–

* Visible only on parting hair.

in every mouse, as first described by Searle (1972). It did not, however, induce the widespread occult type of hair colour loss with pigmented hair tips as seen with *p*-cresol.

In the black male mice, patches of complete hair pigment loss were induced by the topical application of *p*-cresol. The tip of the hair was not exempted, but the process was intermittent at times, some hairs showing only a central band of melanin. Depigmentation of the pigmented epidermis, especially of the tail, was also observed. The *p*-cresol proved to be toxic, the animals dying when large amounts were painted on the skin. Repeated application had a local caustic, erosive effect, not seen in the agouti mice.

DISCUSSION

Lerner (1971) has emphasized that the chemicals that stop melanin production and thereby produce depigmentation are nearly always mono- and dihydroxyl phenyl derivatives or thio compounds. In the phenyl class, activity is dependent on the presence of a hydroxyl group and a non-polar group at diametrically opposite points on the benzene ring. It is this *para* positioning which mimics the structural patterning of tyrosine, the amino acid building block for melanin. Apparently such stereochemical pseudo-identity explains in part the capacity of these compounds to enter and interfere with the replicative biochemical processes in the formation of melanin.

In our search for the component in laundry-ink which depigmented the hairs of mice, *p*-cresol was the likely suspect, since it shows the requisite hydroxyl and methyl groups in the 1,4 positions, on a benzene ring. Furthermore, Brun, in 1961, showed that 1% of *p*-cresol in vaseline induced local depigmentation in guinea-pigs. Indeed, it proved to be the specific depigmenting chemical in the laundry-ink, its stereo-isomers, *meta*- and *ortho*-cresol being without effect.

The original observation that the topical application of Aero® laundry-ink induced localized bands of white hair suggested that the ink was acting upon the follicular melanocytes at a critical period in the hair growth cycle. In this regard, the experiments of Searle (1972) and Haddow (1973) are most instructive. They were able to induce whorls, bands, targets and other patterns of depigmentation or colouring in mice and rats by the topical application of 8-hydroxyquinoline and the feeding of an alloxazine dye respectively. In each instance, the depigmentation and the hair colouring were dependent on the chemicals acting on the hair follicle during the initial sensitive period of the hair growth cycle. Such waves of new growth constantly swirl over the skin of these animals, accounting for the dramatic patterning produced by depigmenting or colouring the hair at the advancing edge. A similar sensitivity to radiation (Potten, 1970; Epper, Houssay & Sancho, 1971) is seen in the mouse during a short period at the beginning of anagen. Plucking or castration, both of which induce a new hair growth cycle, render the animal's follicular melanocytes sensitive to radiation for a brief 2 or 3 days. Only during this period would radiation induce the formation of white hairs.

Thus, in our experiments the hair was plucked and the compounds applied at the critical time in the initiation of a new cycle. This led regularly to depigmentation of the hair. In the agouti mice the exemption of the tip was remarkable (Figs. 1a and 2). Apparently the initial burst of pigment formation in their new hair was not altered by the *p*-cresol applications. The finding of this type of depigmentation was fascinating in that it was inapparent, being disclosed only by parting the hair. The animals retained their phenotypic protective coloration despite the severe chemical attack on their follicular apparatus. Searle (1972) noted pigmented tips on a few of the new hairs in his mice treated with 8-hydroxyquinoline, but only sporadically. In some animals treated with *p*-cresol, the new hair was totally white (Fig. 1b), indicating a more marked complete depigmentation, such as is regularly seen with the use of 8-hydroxyquinoline. Phenol had a similar but far less marked effect. In some mice the toxic effect completely inhibited regrowth of hair in small areas, reminiscent of alopecia areata.

In the clipped areas or in the natural fur, target patterns of white hairs followed the repeated topical application of *p*-cresol or diluted ink. Thus, *p*-cresol reduplicated the spotted, banded white hair originally observed by chance in mice marked with laundry-ink (Shelley & Raque, 1972).

The mechanism of the depigmentation would appear to be melanocidal, since the effect is essentially permanent. New cycles of hair do not show repigmentation. Were it the simple well known tyrosinase inhibition (McGuire & Hendee, 1971), one might anticipate reversibility or even banding of the depigmentation seen in the hair shaft, but this was not ordinarily observed. It seems more likely that, as Riley (1970, 1971) has postulated, tyrosinase acts on certain phenols to form free radical derivatives which initiate lipid peroxidation with consequent permanent melanocyte damage or destruction.

It is significant that the effect that we and also Searle have observed is elicited only by exposure of the melanocyte at a critical time in its reproductive cycle. This is analogous to the effects of radiation where doses must be administered at a similar critical point in the hair cycle to induce depigmentation. In contrast, nitrosourethane depigmentation, recently described by Schoetal (1971), occurs at any site where it is injected, irrespective of the hair cycle.

The striking hair depigmentation we have regularly observed in the CBA/J mouse suggests that this is a good animal for screening the activity of chemicals for possible effects on melanin synthesis. Such compounds should be applied topically at the time of plucking and serially thereafter. Observations should be made on the new hair growth, taking care not to miss the occult depigmentation associated with normally pigmented hair tips. It should be noted that, because of striking species differences in the susceptibility of the melanocyte to chemicals (Lerner, 1971), results cannot be directly transferred to man. Nonetheless, it is interesting to note that the depigmentation of human skin following contact with phenolic germicides (Kahn, 1970) may be patterned, suggesting the possibility of a cyclic variation in melanocyte sensitivity.

ACKNOWLEDGMENTS

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Sweat proteins

D.McEWAN JENKINSON, R.M.MABON AND W.MANSON

Departments of Physiology and Chemistry, The Hannah Research Institute, Ayr

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SUMMARY

The electrophoretic patterns of skin washings from the calf, sheep, goat, pig, horse and man have been compared. It was concluded that protein in these washings was derived mainly from sweat. The amounts of protein lost and the nature of the proteins in the sweat differed markedly between species. The horse had relatively the highest protein loss during active sweating and man the lowest. The potential biological role of these sweat proteins is discussed, including the possibility that they may provide the animal with an outer defence, on the skin surface, against invasion by foreign organisms.

Smith (1890) and Jirka & Kotas (1959) reported the presence of protein in horse sweat, and more recently the nitrogen in cattle skin secretions has been shown to be mainly in the form of a protein or proteins (Jenkinson, Mabon & Manson, 1973). From these results it appeared probable that proteins are normal constituents of mammalian sweat. Free amino acids were not detected in skin secretions from cattle. In contrast, the sweat of man has been reported to contain free amino acids (Hadorn *et al.*, 1967; Morimoto & Johnson, 1967; Liappis & Hungerland, 1972) and substances, probably proteins or mucoproteins, having antigenic activity (Wilkinson *et al.*, 1971).

The present study was undertaken to determine if proteins are present in the sweat of the sheep, goat and pig, and to compare their electrophoretic patterns with those of the cow, horse and of man. The sweat protein composition, if different between species, could reflect different patterns of bacterial growth on the skin surface and perhaps different allergic responses on the skin. Information on such proteins could perhaps also prove of value in the study of sweat gland activity of different species, and possibly also in assessing the nitrogen requirements of some species.

METHODS

Procedure

Sheep and goat. (*a*) *Goat.* Four female British Saanen goats, aged from 2 to 6 years, were kept in an environment of 20°C dry bulb (DB) and 17·5°C wet bulb (WB) on a daily diet of $1\frac{1}{2}$ lb of goat mix with hay *ad lib.* for several weeks before and during the experiment. They were subjected in pairs, on consecutive days, to an environment of 35°C DB and 20°C WB for 6 h. During the experiment the goats were kept under protective hoods in stalls designed to prevent them licking or rubbing the experimental regions. Urine was pumped into closed vessels from a collection funnel, and faeces were collected in polythene bags to prevent contamination. A record of the evaporative loss from the skin was taken from the dorsal surface of each animal throughout the 6 h of the experiment using the ventilated capsule technique of McLean (1963). A large area of skin along the dorsal surface was shaved on both sides of each animal 24 h before entering the climatic chamber. On entry the experimental region was thoroughly washed with cotton wool soaked in 10% methanol, and a skin washing was immediately obtained from two areas, one on each side of the animal from the pre-washed region. A plastic cup 28 cm^2 in area was placed on the skin, 10 ml of distilled water were added and agitated against the skin for 1 min by means of a glass rod. The distilled water was removed with a Pasteur pipette and the samples from the two areas were combined for chemical analysis. The experimental region was re-washed with 10% methanol and 3 h later skin washings were taken from a total of eight areas (four on each side of the animal) from the pre-washed regions using the cup collection procedure. The equivalent pairs of samples on each side of the animal were pooled to give four samples of 20 ml for analysis. The experimental regions were again washed with 10% methanol and 3 h later, at the end of the 6 h period, a further four samples of 20 ml were collected for chemical analysis. The goats were rested for 2 days and then subjected in pairs for 3 h to an environment of 15°C D Band 8·5°C WB. A record of evaporative loss from the skin was again obtained throughout the experiment and skin washings for chemical analysis were collected immediately after entry and after 3 h exposure to the 15°C environment in the manner described above.

(*b*) *Sheep*. The above experiment was repeated using four adult, castrated, male, crossbred Finnish Landrace–Dorset Horn sheep maintained on 3 lb of a complete diet in the control environment of 20°C DB and 17·5°C WB. In this instance, however, two pairs of skin washings were taken at each collection time.

Horse. Skin washings were taken from a clipped area of 60 cm^2 on the neck of each of two thoroughbred horses (a 3-year-old gelding and a 5-year-old mare) and two ponies (adult geldings). The experimental areas on the thoroughbreds were cleaned thoroughly with cotton wool soaked in 10% methanol immediately before 20 min mild galloping, and skin washings were taken immediately afterwards. The skin washings were, in this instance, for practical reasons, obtained by gently rubbing the experimental regions thrice with cotton wool moistened each time with distilled water and finally with dry cotton wool. The entire procedure took approximately 3 min. All four cotton wool pads were then placed together in sealed bottles for chemical analysis. The two horses were transferred to their boxes, fed, watered, and rubbed down. 3 h later the experimental regions were again washed with 10% methanol, and a 'resting' skin wash was obtained 5 h later from the experimental regions in the manner already described. The same procedure was adopted for the two ponies, with the exception that the 'resting' sample was obtained over a 3 h period before exercise and the exercise consisted of a 1 mile walk, a short canter and a further 1 mile walk home.

Man. Skin washings were obtained from a total of five subjects, three men and two women aged from 19 to 37 years. Four subjects, two men and two women, were seated for 3 h in a climatic chamber in an environment of 15°C DB and 8·5°C WB. On entering the room the forehead and both arms of each subject were washed with cotton wool soaked with 50% methanol. Skin washings from the forehead and both arms were immediately taken using the cup collecting procedure already described and the regions were then re-washed. After 3 h in the experimental environment, skin washings were again taken and the areas were rewashed with 50% methanol. The subjects then exercised for 5 min in the experimental environment by stepping up and down onto a stool 1 ft high, and the skin sampling procedure was repeated on the forehead and arms. Each time the samples from the arms were pooled for analysis. In the afternoon of the same day, this same experiment was repeated in an environment of

40°C DB and 19°C WB. A fifth adult male subject was also subjected for 3 h to this environment, except that instead of sitting throughout he underwent the stepping exercise for 5 min every ½ h.

Pig. Skin washings were obtained from two large white pigs immediately after slaughter using the cup collection procedure. In this instance, however, it was not possible to obtain samples from pre-washed regions or to make measurements of the evaporative loss from the skin.

Chemical analysis

All skin washings were concentrated using membrane filters (Amicon UM 20E, Exclusion 10,000–20,000 MW) and adjusted to a volume of 1 ml. The cotton wool pads used to wash the experimental areas on the horse were eluted with water before filtration. In samples from man the concentrates were further washed with 10 ml of distilled water, and the pooled filtrates were tested for the presence of free amino acids using an automatic analyser (JEOL (UK Ltd), London) following the method of Spackman, Stein & Moore (1958). The concentrates were each divided into two; one part was used to determine the quantity of protein present in the residue using the colorimetric technique of Lowry *et al.* (1951), and the other to determine the electrophoretic pattern of the protein or proteins present. In all instances electrophoresis was undertaken on cellulose acetate strips in borate buffer (pH 8·4) at 200 V (1–2 mA) for 20 min, and the bands were stained using Amido black.

RESULTS

Sheep and Goat

Protein was detected in skin secretions from both sheep and goats at 15°C and also at 35°C. This loss of protein from the skin was closely related to sweat gland activity (Fig. 1). The goat lost on average

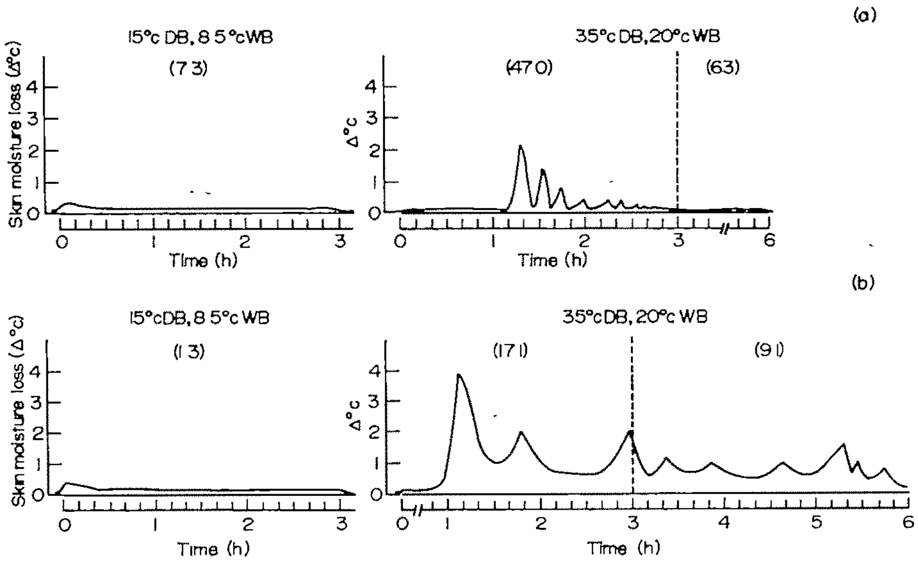

FIGURE 1. Records of cutaneous moisture loss from the back of (a) a goat and (b) a sheep in two different ambient conditions. These patterns occurred in all the sheep and goats studied, and in both species sweating activity was evident in the warm environment but did not occur at 15°C. The goats, however, only sweated for a limited period. The mean protein losses (mg m^{-2} h^{-1}) from the skin over a 3 h period, which are given in parenthesis, reflected sweating activity.

47·0 mg m^{-2} h^{-1} of protein at 35°C during the period of active sweating. This was a significantly greater amount ($P<0·001$) than that lost during a further 3 h exposure to this condition when the sweat glands had fatigued (6·2 mg m^{-2} h^{-1}) or at 15°C (7·3 mg m^{-2} h^{-1}), a temperature at which sweating did not occur. There was no significant difference between the protein losses recorded at 15°C and those found during the second phase of the 6 h exposure to 35°C. The pattern of sweating in the crossbred sheep at 35°C was slightly different, and sweating peaks were observed throughout the 6 h exposure to this condition (Fig. 1). The initial peaks were, however, greater in magnitude and were reflected by a significantly greater protein loss ($P<0·001$) over the initial 3 h period (17·1 mg m^{-2} h^{-1}) than over the second (9·1 mg m^{-2} h^{-1}). This latter protein loss was in turn significantly greater ($P<0·001$) than that found at 15°C in the absence of active sweating.

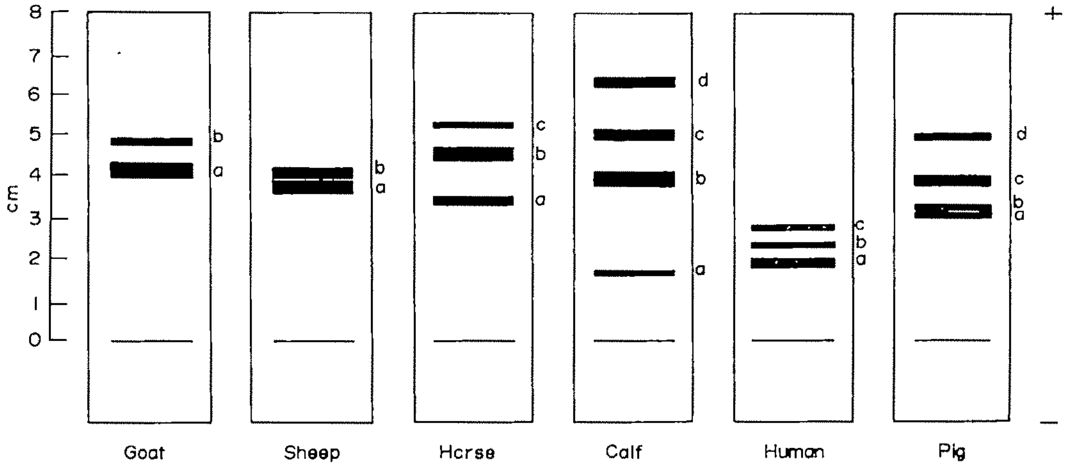

FIGURE 2. Tracings of the electrophoretic patterns of skin washings all treated in exactly the same manner (cellulose acetate strips; 200 V, 1–2 mA, borate buffer for 20 min) from six different species. The diagram illustrates differences in the proteins of the washings, although comparable bands were observed in some instances, e.g. band 'a' in the goat and band 'b' in the sheep.

The electrophoretic patterns obtained from skin washings from the sheep and goats are illustrated in Fig. 2. In both instances, two bands of protein, which ran to mean distances of 4·0 and 4·8 cm in the goat and 3·6 and 4·0 cm in the sheep, were present. In comparison, bovine serum albumin (Sigma (London Ltd) ran to a distance of 3·1 cm.

Horse

After exercise, which caused visible sweating in all four animals and profuse sweating in the thoroughbreds, there was a large increase in the protein loss from a given area of skin (Table 1). The mean losses during a 'resting' period were similar between the thoroughbreds and ponies, and were large compared with those found in the sheep and goats. The greater protein loss from the thoroughbreds than from the ponies, after exercise, was almost certainly due to the fact that they underwent more severe exercise. The electrophoretic pattern obtained from equine skin washings is illustrated in Fig. 2. Three bands of protein running to distances of 3·3, 4·4 and 5·3 cm were observed.

Man

Protein was also present in washings from human skin (Table 2). Subjects resting at 40°C lost about 2·3 mg m^{-2} h^{-1} of protein, an amount which was just significantly greater than that obtained from the same subjects resting at 15°C ($P<0.05$). Exercise at 40°C, which caused profuse sweating on the forehead, however, resulted in a much greater protein loss per unit area than exercise at 15°C ($P<0.001$). At the higher ambient temperature there was a visibly greater sweat output from the forehead than the arm, and a correspondingly greater protein loss from the former region. The protein loss was greater after exercise-induced sweating than after heat-induced sweating, and was apparently related to the severity of the exercise, as indicated by the relatively large loss from the one subject who exercised more severely. The pattern of electrophoresis of human skin washings is illustrated in Fig. 2. Three protein bands running to distances of 1·8, 2·3 and 2·7 cm were observed. No attempt was made at this stage to determine the amino acid composition of the sweat protein or proteins. This protein material, however, accounted for only about 3% of the amino acids present in human skin washings.

TABLE 1. Mean protein output from horse skin (g/m²)

Horse	During a 3 h 'resting' period before exercise	Immediately after exercise	During a 5 h 'resting' period after exercise
1	—	1·23	0·18
2	—	1·57	0·57
3	0·18	0·64	—
4	0·04	0·38	—

TABLE 2. Mean protein output from human skin (forehead and arms)

	Environmental temperature	
	15°C	40°C
Resting for 3 h (mg m^{-2} h^{-1})	1·44	2·28
Immediately after exercise (5 min) (mg/m²)	1·09	7·06
Exercise for 5 min every 30 min for 3 h (mg/m²)	—	65·0*

* One subject.

Most of the amino acids in such washings were present in the free state. The ratios (approximately 97%) of free amino acid to threonine (μmol amino acid/μmol threonine) were as follows: lysine, 0·41; histidine, 0·71; arginine, 0·70; aspartic acid, 1·13; threonine, 1·00; serine+glutamine, 4·64; glutamic acid, 0·88; proline, 0·06; glycine, 2·05; alanine, 1·26; valine, 0·67; methionine, <0·05; isoleucine, 0·27; leucine, 0·31; tyrosine, 0·23; phenylalanine, 0·11.

Pig

Protein was found also in skin washings from pigs, and the pattern of electrophoresis is also shown in Fig. 2. In this species four bands, two of which were closely associated, running to distances of 3·0, 3·2, 3·8 and 4·9 cm were found.

DISCUSSION

The results demonstrate that protein is present in skin washings from sheep, goats, horses, pigs and humans. In the goat at low environmental temperatures, or during the period of sweat gland fatigue which occurs on prolonged heat exposure (Jenkinson & Robertshaw, 1971), the cutaneous protein loss was much less than that observed during active sweating. The protein loss from the skin of the sheep was also closely related to sweat gland activity, although the sweating pattern observed differed from that of the goat. The pattern of sweating is, however, known to vary between different breeds of sheep and goats (Allen & Bligh, 1969). In the horse and man there was an increase in cutaneous protein loss after exercise when visible sweating occurred, and the quantity produced was apparently dependent on the severity of the exercise. The results confirm previous conclusions that protein is present in horse sweat (Smith, 1890; Jirka & Kotas, 1959). Protein has also been found in cattle sweat (Jenkinson, *et al.*, 1973), and it may be concluded from the above evidence that the protein in sheep and goat skin-washings is also mainly derived from sweat. The protein in the secretions from pig skin is likely also to originate from sweat. The results, therefore, support the view that proteins are normal constituents of mammalian sweat and that they occur in male, female and castrated male animals. They also demonstrate, however, that the nature of the proteins in the sweat and the amounts produced vary markedly between species.

The greatest protein loss was observed in the horse. As much as 1·5 g of protein could be lost per square metre of skin during moderate exercise, confirming the findings of previous authors that during exercise there is a considerable protein loss from horse skin (Smith, 1890; Jirka & Kotas, 1959). This could be a contributory factor to the loss of condition of some thoroughbred horses during training. Although present only in small amounts, the protein material in human sweat differed markedly from those in the sweats of the other species. The electrophoretic pattern was consistent and was found in sweat collected after heat exposure or after exercise. Three bands of protein were observed, and it is noteworthy that Wilkinson *et al.* (1971) described the presence of three antigens in human sweat. It seems not unreasonable to postulate, therefore, that the sweat proteins, in some species at least, could have immunological properties. Such proteins could provide the animal with an outer defence, on the skin surface, against foreign organisms and play a part in the development of cutaneous allergic reactions.

Although the protein output in man was small relative to other species, the amount of free amino acid was more than thirty times greater than that of protein in the sweat. The relative amounts of these free amino acids were similar to those described by Hadorn *et al.* (1967). The possibility that these amino acids, or a substantial portion of them, arise from sweat proteins by intervention of enzymes derived from skin bacteria is remote. The absence of significant amounts of free amino acids in cattle sweat (Jenkinson *et al.*, 1973), which is known to be in contact with appreciable numbers of bacteria, lends support to this conclusion. It appears more likely that free amino acids are secreted by the human sweat gland. The small amounts of protein in human sweat could represent an excretory loss from the glands. On the other hand, the different amounts of proteins in the sweat of different species may reflect differences in the rates and modes of secretion. In the horse, the large amount of protein lost during sweating indicates active protein secretion by the glands. The sweat glands of the cow, sheep and goat lose a proportion of the protein content of their lumens (Yang, 1952; Jenkinson & Robertshaw, 1971) during sweating. This must be replaced, and consequently in these species sweating, including protein production, is also likely to be an active process. A passive sweating process, such as that postulated by Alvarez, Hahn & Johnson (1970), or an osmotic mechanism, involving a protein in the lumen (possibly hydrophilic) which is replaced by the secretory cells during intervals between sweating, seem much less likely alternatives. The protein in sweat will provide a medium for bacterial growth on the skin surface and differences in sweat proteins between species may also result in different cutaneous bacterial populations and could perhaps reflect differences in resistance to cutaneous disorders. It is evident, however, that further investigation of the nature of the sweat proteins in different species is required before their role in the biology of the skin and skin surface can be determined.

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Pharmacology and Treatment

Azathioprine in the treatment of muco-cutaneous pemphigoid

VINAY K.DAVE AND C.F.H.VICKERS

Department of Dermatology, Liverpool Royal Infirmary, Liverpool

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Four patients with muco-cutaneous pemphigoid were treated with azathioprine. Satisfactory control of the cutaneous lesions was maintained in two patients, in one of them with concomitant use of a corticosteroid. Oral ulceration was less frequent in three patients, and in two patients with ocular involvement visual impairment did not progress. Although the various complications of azathioprine therapy need to be considered, this drug may significantly improve the prognosis regarding vision in patients with ocular changes otherwise totally unresponsive to therapy.

Immunosuppressive drugs, notably methotrexate and azathioprine, have been used successfully in the treatment of pemphigus and bullous pemphigoid (Lever & Hashimoto, 1969; Jablonska, Chorzelski & Blaszczyk, 1970; Greaves *et al.*, 1971). Histologically, the subepidermal bullae in muco-cutaneous pemphigoid cannot be distinguished from those of bullous pemphigoid. Recent immunofluorescence studies in muco-cutaneous pemphigoid have shown the presence of tissue-fixed basement membrane zone antibodies similar to the basement membrane zone fluorescent staining characteristic of bullous pemphigoid, suggesting a relationship between the two diseases (Bean *et al.*, 1972). A trial of immunosuppressive therapy appears justifiable in muco-cutaneous pemphigoid on this basis and also because systemic corticosteroid therapy may control the cutaneous and oral lesions but does not influence the progressive ocular changes. Azathioprine was selected since it is an effective immunosuppressant and its metabolite, 6-mercaptopurine, has an additional non-specific anti-inflammatory action (Hersh, Wong & Freireich, 1966).

CLINICAL CASES

The clinical diagnosis of muco-cutaneous pemphigoid in four patients was confirmed by histological examination of skin biopsy specimens. Prior to and during the course of therapy with azathioprine, full blood counts, urine analysis and liver function tests were undertaken at regular intervals. Some of the clinical details and the results of the therapy are shown in Table 1.

Case 1

Initially there was a moderate response in this patient to treatment with prednisolone 30 mg daily, though after 3 months he developed steroid-induced diabetes. Five months after combined treatment

TABLE 1. Clinical details of patients treated with azathioprine

Case no.	Age and Sex	Clinical features	Total duration of disease	Previous treatment	Dosages (daily)		Duration of treatment with azathioprine	Results		Complications
					Azathioprine	Corticosteroid		Skin	Mucous Membrane	
1	62M	Sudden hoarseness and dysphagia, bulbar-lid conjunctival adhesions right eye, ulceration oral mucosa, epiglottis and forehead	4 years 2 months	Prednisolone 30 mg daily Contact lenses Methylcellulose eye drops	50–150 mg	Prednisolone 5–10 mg (5 months)	1 year 11 months	Lesions cleared	Ocular changes controlled. Occasional oral ulceration	Steroid-induced diabetes
2	62M	Blisters on chest, back and forearms. Bullae—larynx. No ocular lesions	3 years 6 months	Prednisolone 240 mg daily Triamcinolone 4–48 mg daily	150–100 mg	Triamcinolone 2 mg	2 years 9 months	Lesions cleared	Occasional oral ulceration	Steroid-induced diabetes and mild diabetic retinopathy in both eyes
3	63M	Bullae on the limbs, oral mucosa and penis with scarring and milia formation. Intermittently has sub-conjunctival bullae	6 years 6 months	Prednisolone 40 mg daily Dapsone 100 mg daily (for 1 year) Betamethasone 1–3 mg daily	100–200 mg	Betamethasone 1–12 mg	Separately 1 year and 3 months	Part controlled	Ocular bullae healed. Occasional oral ulceration	Steroid-induced diabetes
4	50F	Recurrent blistering 6 years on side of face. No mucous membrane lesions apart from occasional small ulcers in the mouth	6 years	Dapsone 100 mg daily 150 mg (for 6 weeks—no improvement). Prednisolone 15–30 mg daily—no effect	150 mg	—	2 months	Temporary improvement but relapse while still on azathioprine	—	—

with azathioprine and prednisolone was instituted, the latter was discontinued as no further oral, laryngeal or cutaneous ulcers had occurred for 2 months. On a maintainance dose of azathioprine 100 mg daily, he was symptom-free for about a year and then developed acute conjunctivitis and iritis in both eyes. There was a gradual response to an increased dose of azathioprine 150 mg daily and methylcellulose eye drops.

Case 2

The patient was first seen at the clinic with bullae on the trunk as well as in the pharynx and larynx. Though there was an initial response to high dosage corticosteroid therapy, steroid-induced diabetes complicated his management and the disease was unsatisfactorily controlled for the next 3 months. A clear response was seen 3 months after the start of combined therapy with azathioprine and triamcinolone (with only occasional cutaneous blistering). Six months later the disease had remitted and remained quiescent until his death a year later of myocardial infarction.

Case 3

Initially this patient proved unresponsive to large doses of prednisolone, to dapsone and to betamethasone. He was maintained on azathioprine 150–200 mg and betamethasone 1 mg daily for a year, after which a severe relapse of the disease necessitated an increase in the dose of betamethasone to 12 mg daily. He was then found to have hepatosplenomegaly, and despite extensive investigations (by Professor D.J.Weatherall), including a liver biopsy, its cause has not yet been established. Azathioprine therapy was discontinued and he was maintained on betamethasone 1 mg daily for 3 months, during which he continued to produce occasional cutaneous bullae. He then had a relapse with extensive bulla formation on the limbs, trunk and buccal mucosa, and a subconjunctival bulla in the right eye. Treatment with azathioprine 100 mg daily was resumed (maintaining the dose of betamethasone at 1 mg daily); within 48 h the erythematous areas, which sometimes precede bulla formation (Lever, 1965), on the trunk and limbs had cleared. The bulla in the right eye also subsided rapidly. The disease is now moderately well controlled by azathioprine 100 mg and betamethasone 2 mg daily.

Case 4

From 1966 this patient has had bulla formation extending from both temples to the sides of the neck, and eventually producing bilateral pigmentation and scarring. A short trial of azathioprine produced a temporary improvement for 2 months. The blistering recurred while the patient was still on the drug and she discontinued taking it.

DISCUSSION

Azathioprine, a purine analogue, produces intercellular release of 6-mercaptopurine in the body (Elion, 1972) and inhibits both DNA and RNA synthesis. In general, the purine analogues are most effective in suppressing antibody formation; proliferation and differentiation are more sensitive to the action of these drugs, than are other stages of the immune response (Makinodan, Santos & Quinn, 1970). The main side-effect is bone marrow depression; however, additional use of a corticosteroid counteracts the leukopenic effect (White, Cameron & Trounce, 1966). Long term use of azathioprine predisposes to infections caused by the cytomegalic virus and various fungi and yeasts. The other toxic effects are hepatitis and increased susceptibility to development of neoplasia (Woodruff *et al.*, 1969). The incidence of malignant tumours in graft recipients maintained on immunosuppressive therapy is higher than in the general population; in one series it was 5% (Penn, Halgrimson & Starzl, 1971). Azathioprine therapy has produced no significant side-effects in our patients. When occasional episodes of leukopenia or thrombocytopenia were noted, the drug was temporarily withdrawn.

Satisfactory control of the cutaneous lesions by azathioprine was maintained in only two patients (Cases 1 and 2). The striking response to azathioprine of the acute erythematous areas which developed in Case 3, and of keratoconjunctivitis in Case 1 may well be due to its anti-inflammatory action. Oral ulceration was less frequent during azathioprine therapy in Cases 1, 2 and 3.

Despite the use of various local measures, such as sub-conjunctival injection of a corticosteroid suspension, instillation of corticosteroid drops, and plastic surgery, including corneal transplantation, the most severe sequela in muco-cutaneous pemphigoid is blindness. In a series of eighty-one patients described by Hardy *et al.* (1971), the eyes were involved in sixty-two patients; twenty-one of these became blind, seventeen in both eyes. The hazards of long-term cytotoxic therapy must be balanced against the grave prognosis regarding vision in patients with muco-cutaneous pemphigoid. Our series of cases is small and spans a relatively short period of time in a disease with a protracted course liable to long periods of spontaneous remissions. No definite conclusions can be drawn regarding the value of azathioprine, but its use as an alternative to high dosage corticosteroid therapy is suggested, as in our patients, apart from controlling the cutaneous and oral bulla formation, it seemed effectively to control the eye lesions and may, therefore, prevent blindness.

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Development and evaluation of ointment and cream vehicles for a new topical steroid, fluclorolone acetonide

T.MALONE, J.K.HALEBLIAN, B.J.POULSEN AND K.H.BURDICK

Institutes of Pharmaceutical Sciences and Clinical Medicine, Syntex Research, Palo Alto, California 94304, U.S.A.

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SUMMARY

Several cream and ointment vehicles were developed for a new topical steroid, fluclorolone acetonide. The relative activity of the drug in these formulations was evaluated by vasoconstrictor assays and by release studies. Enhanced drug release and superior vasoconstrictor activity were demonstrated by fluclorolone acetonide when prepared in vehicles in which it was entirely solubilized. Furthermore, the correlation found between solubility, release and *in vivo* response demonstrated the usefulness of *in vitro* release studies in predicting vehicle efficacy. The technical aspects of development and evaluation of ointment and cream vehicles for this steroid are discussed.

During the formulation of a drug for topical application, one must consider several aspects of vehicle composition in order to obtain a superior final product: a physically stable and cosmetically acceptable vehicle, which also can provide an environment in which the drug is stable, and from which it is readily released when placed in contact with skin.

Bio-availability of a topical drug is an important consideration, because the ease or difficulty with which the drug penetrates will significantly affect the therapeutic results obtained. Improper formulations can render a potentially active topical drug essentially ineffective.

This study was designed to characterize the release of a new topical steroid, fluclorolone acetonide,* from different ointments and creams, and to determine the effect of vehicle composition on release and vasoconstrictor response.

Fluclorolone acetonide is a potent corticosteroid synthesized by Bowers (1965), with a topical activity 105 times that of cortisol, using the rat ear assay (Dorfman, 1970). When tested using the growth inhibition of fibroblasts as an index of its activity, its potency was 320 times that of cortisol (Berliner *et al.*, 1970). Specific chemical features (Fig. 1) contributing to fluclorolone acetonide's superior potency are a double bond between carbons 1 and 2, a fluorine atom at the 6α position, a unique 9α,11β-dichloro addition and a 16,17-acetonide group. As illustrated by the standard alcoholic vasoconstrictor test (Moore-Robinson & Christie, 1970), these additions make this corticosteroid more potent than betamethasone-17-valerate, fluandrenolone, beclomethasone propionate, flucortolone caproate and flumethasone pivalate.

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* Chemical name: 9α,11β-dichloro-6α-fluoro-16α,17α,21-trihydroxypregna-1,4-diene-3,20-dione 16,17-acetonide. USAN name: flucloronide.

However, in order to maximize the therapeutic effects of this relatively more potent and sophisticated corticosteroid, one must also rigourously apply various physical pharmaceutical principles to vehicle design. Ostrenga *et al.* (1971) have shown on theoretical grounds that for release from topical vehicles, a given drug will be released more rapidly if the drug is completely solubilized (assuming that other parameters such as viscosity remain constant). If the drug is in solution, the vehicle is homogeneous, and sink conditions prevail. The amount of drug released per unit area, Q_1 is given by equation 1 (Barrer, 1951)

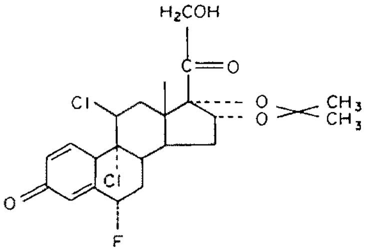

FIGURE 1. Structure of fluclorolone acetonide.

$$Q_1 = hC_0\left[1-\frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)^2}\exp\left(\frac{-D(2m+1)^2\pi^2t}{4h^2}\right)\right] \quad (1)$$

where D is the diffusion coefficient for the drug in the vehicle, h is the thickness of the vehicle, C_0 is the initial drug concentration, m is an integer, and t is time. For small t, when only the first term of the sum in equation 1 is significant, the relationship simplifies to:

$$Q_1 \simeq 2C_0(Dt/\pi)^{\frac{1}{2}} \quad (2)$$

However, if a substantial fraction of the drug is in the form of a suspension, it has been shown that (Higuchi, 1961)

$$Q_2 = (ZA-C_S)\left[\frac{Dt}{1+\frac{2(A-C_S)}{C_S}}\right]^{\frac{1}{2}} \quad (3)$$

where Q_2 is the amount of drug released, A is the concentration of drug, C_S is the solubility of the drug in the vehicle. If $A \gg C_S$, Equation 3 simplifies to

$$Q_2 \simeq (2ADC_St)^{\frac{1}{2}} \quad (4)$$

From equation 2 and 4, one can obtain the following relationship

$$Q_1 = \left[\frac{2A}{\pi C_S}\right]^{\frac{1}{2}} Q_2 \quad (5)$$

when C_0 is set equal to A in the case of Q_1. When $A \gg C_S$ the proportionality factor $(2A/\pi C_S)^{\frac{1}{2}}$

becomes greater than one, subsequently $Q_1 > Q_2$. Thus, at a given concentration, release is always faster from a vehicle in which the drug is completely solubilized. The goal in topical vehicle formulations is to maximize the release rate without unfavorably altering other factors which affect the finished product.

MATERIALS AND METHODS

Formulations

Three ointment and three cream formulations containing 0·025% (w/w) fluclorolone acetonide were prepared for this study. These formulations were selected in order to evaluate correlations between the quantity of drug solubilized in the formulation and its availability as judged by drug release and *in vivo* vasoconstriction tests. Accordingly, the formulations represent the following degrees of solubilization of fluclorolone acetonide: (1) poorly dissolved (suspension); (2) optimal solubilization (saturated solution); and (3) oversolubilization (solubilizer in excess of that required to completely dissolve the steroid). The qualititive composition of these ointments and creams are given in Tables 1 and 2, respectively.

TABLE 1. Qualitative composition of ointments

Ointment	Discontinuous (solubilizer) phase	Continuous matrix phase	Emulsifiers	Approx. percent drug solubilized
1	None	Petrolatum	None	0 0
2	Propylene glycol Propylene carbonate	Petrolatum	Amerchol CAB*	100·0
3	Propylene glycol	Petrolatum	Anhydrous lanolin	100·0

* American Cholesterol Products, Inc., Amerchol Park, Edison, New Jersey.

TABLE 2. Qualitative composition of creams

Cream	Discontinuous phase	Continuous (solubilizer) phase	Approx. percent drug solubilized	Processing method
1	Vanishing type cream		4·0	Drug dispersed in placebo cream
2	Vanishing type cream		4 0	Drug dissolved during processing
3	Polyethylene Glycol 4000 Fatty alcohol	Glycerin Propylene glycol	100·0	Drug dissolved during processing

In vitro *release of ointments*

Each 3 g sample of ointment prepared with ^{14}C-labelled fluclorolone acetonide was filled into a teflon cell having a diameter of 3·0 cm and a depth of 0·4 cm. A disc of filter paper,* dampened with water, was applied to the surface of the ointment to avoid the introduction of air bubbles. A teflon ring was clamped over the filter paper to hold the ointment in place.

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* Whatman Filter No. 54.

Each teflon cell containing the ointment was placed upside down on a wire-gauze platform (Fig. 2) in a beaker containing 60 ml of water. The cells were placed upside down to prevent diffusion of the mineral oil fraction of the petrolatum into the receptor phase. A stirring blade (7·5 × 2·5 cm) was lowered halfway into each receptor phase. The receptor phase was stirred at 50 rev/min by a multiple stirring apparatus.* The release apparatus was kept in a water bath at 25°C. A 2 ml aliquot of the water receptor was removed periodically from each beaker, evaporated to dryness (to avoid quench effects) and mixed with 15 ml scintillation fluid. The fluid consisted of 0·26 g of 1,4-bis[2-(4-methyl-5-phenyloxazoylyl)] benzene,† 208 g naphthalene,‡ 1000 ml dioxane,§ 1000 ml of scintillation grade toluene,** and 600 ml methanol.† The samples were counted on a liquid scintillation counter.†† The receptor phase was quantitatively replenished with pure water at each periodic sampling.

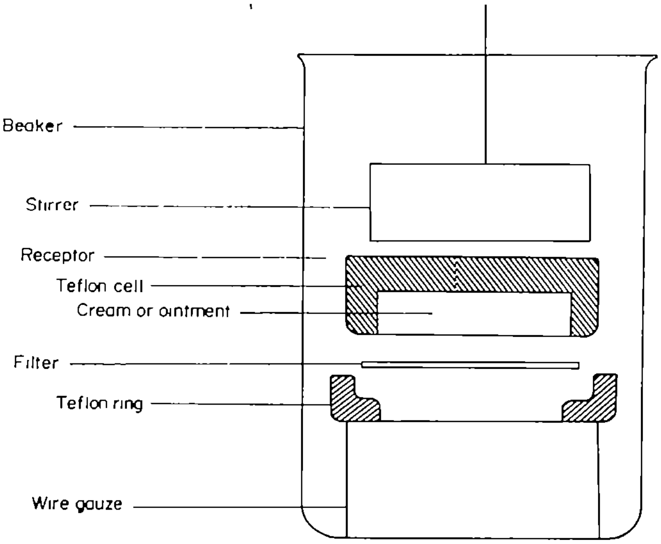

FIGURE 2. System used for release studies.

In vitro *release of creams*

The same procedure was followed as for the ointment study. However, in studying release from creams, 100 ml of isopropyl myristate‡‡ was employed as the receptor phase (Poulsen *et al.*, 1968). In addition, 1 ml aliquots of receptor phase were withdrawn and replenished periodically, and directly combined with 15 ml of the scintillation fluid for counting.

Vasoconstrictor studies

The vasoconstrictor studies were performed using the method developed by McKenzie & Stoughton (1962) and modified by Burdick, Poulsen & Place (1970). The different formulations were applied to healthy subjects who had not participated in any study using steroids for at least 4 weeks before testing. Each subject was treated with each formulation on two randomized sites on each forearm, for a total of sixteen sites, using 3 mg of preparation per 7 mm square site punched in double-adhesive-coated tape.* After the application of the formulations, one forearm was covered with a plastic film.† 6 h after application the forearms were washed and were not covered again. The sites responding were recorded at 8, 24 and 32 h after application.

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* Phipps and Bird, Inc., Richmond, Virginia; † Arapahoe Chemicals, Div. Syntex Corp., Boulder, Colorado; ‡ J. T. Baker Chemical Co., Phillipsburg, New Jersey; § Mallinckrodt Chemical Works, St Louis, Missouri; ** Matheson Coleman and Bell, Los Angeles, California; †† Nuclear Chicago Corp., Model #720, Des Plaines, Illinois; ‡‡ General Aniline and Film Corp., New York.

RESULTS

Ointments

Two types of ointment formulation containing 0·025% (w/w) fluclorolone acetonide were investigated. Three ointments were studied. The first was a conventional ointment in which the fluclorolone acetonide crystals, which measure from 30–50 μm, were suspended in petrolatum. The second and third were vehicles in which the drug was completely solubilized in the internal or the discontinuous phase, which in turn was dispersed in petrolatum with the aid of emulsifiers.

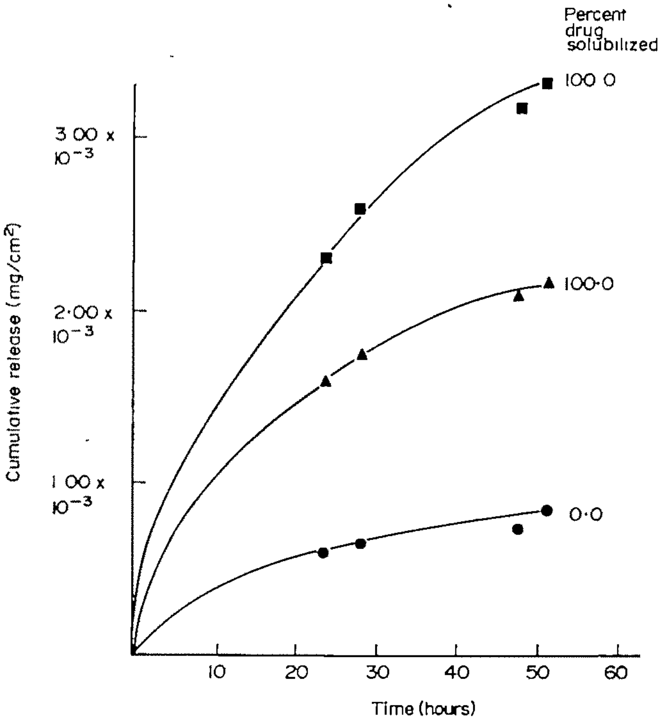

FIGURE 3. Fluclorolone acetonide release from various ointments into water at 25°C. ●, ointment 1; ▲, ointment 2; ■, ointment 3.

The release curves given by the different ointments are shown in Fig. 3 along with estimates of the percentage of drug solubilized. The percentage of drug solubilized for each formulation was estimated by the method of Ostrenga *et al.* (1971).

From these curves it can be seen that the lowest release was given by the ointment in which the drug was in suspension. The release rates from ointments 2 and 3, in which the drug was completely solubilized, followed a square root of time dependency. Ointment 2, in which there was an excess of solubilizer (enough to solubilize approximately twice the concentration of fluclorolone acetonide), had a release profile less than that for ointment 3, in which the exact amount of solubilizer (enough to solubilize 0·025% fluclorolone acetonide) was present.

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* Blenderm®. † Saran Wrap®.

The ointments were also tested *in vivo* by a modified human vasoconstriction assay (Burdick *et al.*, 1970). These results are given in Table 3. The percentage of sites responding (vasoconstriction) at 8, 24, and 32 h were recorded for both open and occluded applications. Since the time intervals chosen for the evaluation of responses were arbitrary and since each steroid gives a unique period of peak response (Burdick, 1971), the column in Table 3 recording the 'total' percentage of sites responding over the entire reading interval seemed to be the best measure of response or efficacy. While excess solubilizer was observed to depress the *in vitro* release of solubilized ointments, the two solubilized vehicles were shown to be approximately equal. Both were clearly superior to the suspension. The comparative results in terms of solubility, release and *in vivo* response are summarized in Table 4.

TABLE 3. Vasoconstriction results for various 0·025% fluclorolone acetonide ointments

		Open application				Occluded application			
		% sites responding at				% sites responding at			
Ointment	Approx. percent drug solubilized	8 h	24 h	32 h	Total	8 h	24 h	32 h	Total
1	0	38	8	9	55	43	6	5	54
2	100	80	27	22	129	77	27	23	127
3	100	72	29	21	122	86	35	20	141

TABLE 4. Comparative solubility, *in vitro* release and vasoconstriction of 0·025% fluclorolone acetonide ointments

			Human vasoconstriction: total score, sites responding	
Ointment	Approx. percent drug solubilized	*In vitro* release: total release at 51 h γ/cm^2	Open	Occluded
1	0	0·72	55	54
2	100	2·08	129	127
3	100	3·24	122	141

Creams

Two types of cream formulation containing 0·025% (w/w) fluclorolone acetonide were investigated. Three creams were studied. In composition, creams 1 and 2 were identical formulations. However, in cream 1 the fluclorolone acetonide 30–50 μm crystals were dispersed into the placebo cream as the final step. In contrast, in cream 2, fluclorolone acetonide was dissolved in the cream during the manufacture of the cream. Both creams are of the vanishing type, containing (in addition to fluclorolone acetonide) stearic acid, propylene glycol, sorbitan monostearate, sorbitan mono-oleate, polyoxyethylene sorbitan monostearate, citric acid, methyl paraben, propyl paraben and water (75%).

Since the major component of this vehicle is water, very little fluclorolone acetonide can be dissolved in this cream. Cream 3 (FAPG® cream) is a new topical vehicle, which appears to be particularly well suited as a delivery system for poorly soluble compounds, such as topical corticosteroids. This vehicle, in which fluclorolone acetonide is solubilized, may be described as a two phase system in which a continuous, solubilizing phase is distributed in a solid matrix of fatty alcohol and high molecular weight polyols. This vehicle has been termed a 'cream/gel', since it combines the physical properties of gels with the cream-like appearance and consistency of emulsion systems. Because it is anhydrous this cream does not require preservatives, many of which have been shown to be potential sensitizers, and thus it provides an optimal cream base for fluclorolone acetonide.

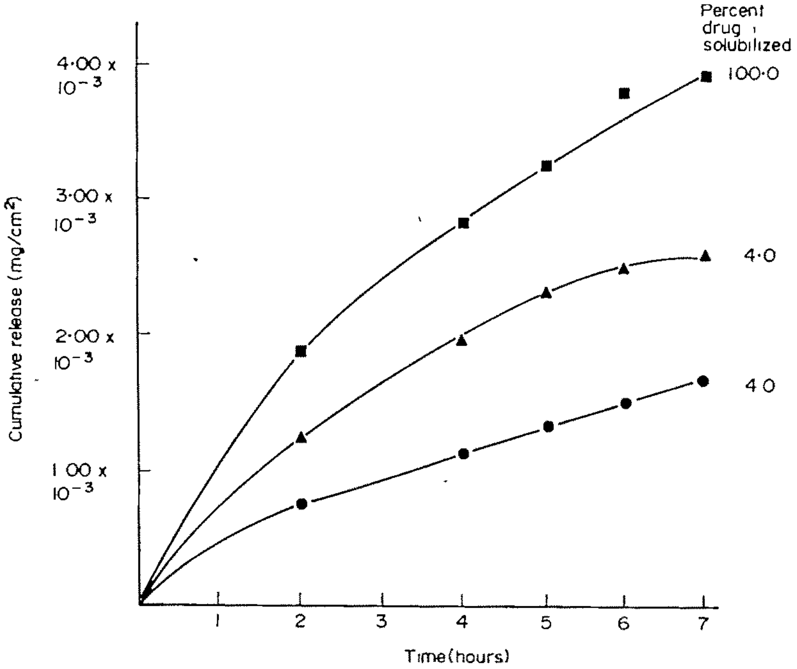

FIGURE 4. Fluclorolone acetonide release from various creams into isopropyl myristate at 25°C. ●, cream 1; ▲, cream 2; ■, cream 3.

TABLE 5. Vasoconstriction results for various 0·025% fluclorolone acetonide creams

Cream	Approx. percent drug solubilized	Open application: % sites responding at 8 h	24 h	32 h	Total	Occluded application: % sites responding at 8 h	24 h	32 h	Total
1	4·0	11	5	9	25	25	11	11	76
2	4·0	30	8	6	44	79	20	17	116
3	100·0	28	23	18	69	73	40	34	147

The release curves given by these creams are shown in Fig. 4, accompanied by the estimated percentage of drug solubilized. Creams 1 and 2, in which the drug is in suspension, yield release profile less than that given by cream 3, in which the drug is in solution.

These creams were tested in the same *in vivo* assay as the ointments. The results are given in Table 5. From this it can be seen that, in terms of rank order, the *in vivo* vasoconstriction response for both open and occluded application correlates with percent drug solubilized in the vehicle and with *in vitro* release rates.

DISCUSSION

The results obtained in this investigation clearly demonstrated that superior release and vasoconstrictor activity of fluclorolone acetonide in both cream and ointment vehicles were positively correlated with the degree of solubilization of this corticosteroid. The comparative results for stability and release for both ointments (Table 4) and creams (Table 6) show that release is faster from a vehicle in which the drug is completely solubilized. This relationship is in agreement with the theoretical considerations (equation 5) discussed in the introduction.

TABLE 6. Comparative solubility, *in vitro* release and vasoconstriction of 0 025% fluclorolone acetonide creams

Cream	Approx. percent drug solubilized	*In vitro* release: total release at 7 h γ/cm^2	Human vasoconstriction: total score, sites responding	
			Open	Occluded
1	4·0	1 72	25	76
2	4·0	2·68	44	116
3	100·0	3·96	69	147

These results also agree with the work of Poulsen *et al.* (1968) which indicated that vehicles which lack sufficient solubilizer yield lower release of a poorly soluble drug such as a corticosteroid. However, when an excess (more than that needed to dissolve the steroid) of solubilizer was present (ointment 2) release of steroid was shown to decrease. This decrease was attributed to the decrease in partition coefficient with increasing solubilizer concentrations.

The significance difference demonstrated both in release profiles and in vasoconstrictor responses for creams 1 and 2, which are identical in composition, can also be attributed to drug solubilization. This difference in release is due to difference in the particle size of the suspended fluclorolone acetonide (Barret *et al.*, 1965; Haleblian *et al.*, 1971). Though identical in formulation composition, these creams differ in their methods of manufacture. In cream 1, fluclorolone acetonide particles of 30–50 μm were dispersed in the finished placebo cream base and no significant changes in particle size of suspended drug occurred. In cream 2 the drug was dissolved in the hot propylene glycol during manufacture. When such a formulation cools to room temperature, gradual precipitation of the drug occurs, since the drug is poorly soluble in the vehicle at room temperature. However, the time for equilibration to be reached may extend over several months. Since cream 2 was freshly prepared for all studies, it is expected that it had not reached equilibrium during the experiments, and thus had a solubility greater than the theoretically calculated 4%.

In conclusion, a positive correlation has been found between solubility, release and *in vivo* response. Furthermore, this correlation demonstrates the usefulness of *in vitro* release studies in predicting vehicle efficacy.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to W.Hafferl and A.Harry of Syntex Research, Institute of Organic Chemistry, for supplying the radioactive tracer, and S.Cobner, R.Kelley and C.Northway of Syntex Research, Institute of Clinical Medicine, for performing the vasoconstrictor studies.

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The clinical evaluation of a new topical corticosteroid, clobetasol propionate

AN INTERNATIONAL CONTROLLED TRIAL*

C.G.SPARKES AND LYN WILSON

Clinical Research Unit, Glaxo Laboratories Ltd, Greenford, Middlesex

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SUMMARY

A new topically active corticosteroid, clobetasol propionate, has been investigated in the laboratory and in dermatological clinics to determine the best formulation for clinical use. Clinical trials involving 1150 patients were undertaken. In patients with bilateral psoriasis or eczema, clobetasol propionate, at a strength of 0·05%, was compared with betamethasone 17-valerate ointment and cream, fluclorolone acetonide ointment and cream and fluocinonide (FAPG). The new steroid was shown to be effective therapeutically, especially in psoriasis. Each comparison in the trial was analysed independently and the following statistically significant differences were demonstrated.

Psoriasis

Clobetasol propionate ointment > fluocinonide ($P<0·01$).
Clobetasol propionate ointment > fluclorolone acetonide ($P<0·05$).
Clobetasol propionate cream > fluocinonide ($P<0·05$).
Clobetasol propionate cream > fluclorolone acetonide ($P<0·05$).

Eczema

Clobetasol propionate ointment > fluclorolone acetonide ($P<0·01$).
Clobetasol propionate cream > fluclorolone acetonide ($P<0·05$).

A number of methods of screening corticosteroids for topical activity have been described. These include the fibroblast inhibition test (Berliner & Nabors, 1967), inhibition of the inflammatory response induced in rats' ears by the application of irritants (Dorfman, 1970), inhibition of mitosis (Fisher & Maibach, 1971; Marks, Pongsehirun & Saylan, 1973), and the vasoconstriction test (McKenzie & Stoughton, 1962). Of these, only the vasoconstriction test has stood the test of time, being both practical for screening large numbers of compounds and reliable, giving a reasonable prediction of therapeutic activity. That this was so, was first shown by McKenzie & Stoughton (1962) and has been confirmed by subsequent clinical experience (Williams *et al.*, 1964; Munro, Wilson & Rhodes, 1967).

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* A list of the dermatologists who took part in the trials appears on page 202.

Other screening tests have shown inconsistencies. For instance, inhibitory values obtained for some steroids in the Berliner test do not correlate with activity in the clinic; potency figures in the Berliner test for betamethasone valerate, triamcinolone acetonide and fluclorolone acetonide are 36, 156 and 320 respectively (Berliner *et al.*, 1970), yet it has not been demonstrated that these three compounds have such widely differing activity in normal use, or even that they are ranked in this order of efficacy (Rhodes *et al.*, 1972; Harman *et al.*, 1972; Wilson, 1973; Williams *et al.*, 1964).

In the past few years a large number of steroids have been synthesized and screened for possible topical activity using the McKenzie vasoconstriction test. Using this method, clobetasol propionate (21-chloro-21-desoxybetamethasone-17-propionate), a compound with a vasoconstriction index of 19 (cf. betamethasone valerate 3·6) was selected for further study in the laboratory and the clinic.

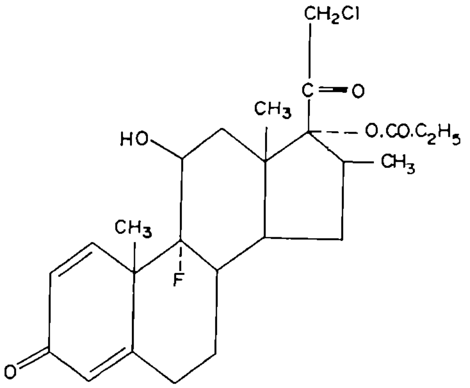


FIGURE 1. Clobetasol propionate.

Bio-availability of steroids from their vehicles is now recognized to be of great importance, thus in preliminary investigations clobetasol propionate was tested in various bases, in strengths up to 0·1%. These studies indicated that the optimal strength lay between 0·025% and 0·05%, and this paper reports the subsequent trials carried out.

METHODS

Patients were included if they had bilateral lesions of eczema or psoriasis which, at the start of the trial, were judged to show little or no difference in clinical severity between the two sides.

The preparations to be compared were allocated at random to left or right sides and the identical looking tubes were labelled accordingly. Neither the clinician nor the patient was aware of their distribution. Patients were asked to treat their lesions at least twice a day, but more frequently if the clinician so desired, either with or without polythene occlusion. Some patients were in hospital, but the majority were out-patients.

Assessment of the response to treatment was made at, or as near as possible to, 7 days after the start of the trial. The lesions were rated as healed, improved, static or worse; in addition, the clinician was asked to record whether the response had been better on one side or the other, or if the lesions were still indistinguishable. In some of the comparisons patients were asked if they experienced any sensation on application of the preparations.

The trial code was held by a trial co-ordinator. As each patient completed the trial the clinician returned the completed record card to the trial centre. Here the card was scrutinized by one co-ordinator, who was not in possession of the code, to ensure that it is was correctly completed and that a clear choice was recorded by the clinician. If there was any doubt about any detail the report was either returned for clarification or excluded from the trial.

The card was then decoded by a second co-ordinator. In each case both objective (by clinician) and subjective (by patient) assessments were made. Differences between these two assessments were extremely rare, so that all analysis of data that follows is based only upon the clinicians' choice. As each card was decoded the result was plotted on a sequential analysis design, as described by Armitage (1960); a choice for one or other treatment being termed a preference, and an equal response (or lack of response) a tied pair. If the preference line met a boundary assigned to one or other treatment it indicated a statistically significant difference in favour of that treatment. Boundary lines were set at two places, such as to indicate a significance of $P<0{\cdot}05$ and $P<0{\cdot}01$. Results were plotted sequentially as they were decoded, and this allowed the trial to be stopped after treating the minimum number of patients. This method analyses preferences only; further data are presented as histograms (Figs. 2 and 3), where the number of choices may be larger than appears in the graphs, as they include a number of patients already under study when the decision was made to stop.

Clobetasol propionate was formulated in both cream and ointment bases. In both bases propylene glycol was used as the solvent to provide optimum conditions for the release of the steroid. Formulations of clobetasol propionate were compared with fluclorolone acetonide, fluocinonide and betamethasone valerate. All three control steroids were used in their marketed formulations (Topilar, Metosyn and Betnovate respectively). Two of these (fluclorolone acetonide and betamethasone valerate) are available in both cream and ointment bases. In these cases, clobetasol propionate ointment was compared with the control ointment and clobetasol propionate cream with the control cream. However, fluocinonide is only available in one base (FAPG), which has been said to have the properties of both a cream and an ointment (Portnoy & Sarkany, 1972). Consequently, this preparation was compared with both clobetasol propionate ointment and cream. In view of the nature of the FAPG base it was considered possible, though unlikely, that there might be a residue of cream, ointment or FAPG left on the skin at the time of assessment and that this might give the clinician a clue as to the identity of the materials, thus introducing a bias into the trial. In order to eliminate this possibility all patients in all sections of the trial were asked not to use the preparations on the day they returned to their doctor for assessment. A card supplied to each patient gave this instruction and also asked them to wash their hands after using the first preparation, before applying the second.

RESULTS

Preliminary investigations

Preliminary investigations were carried out to confirm the laboratory predictions of the ideal bases for release of the steroid and to establish the optimal concentration of clobetasol propionate.

Bases. The topical vasoconstrictor activity of clobetasol propionate formulations was measured using a modification of the McKenzie assay described by Busse *et al.* (1969).

For the ointments, it was found that maximum vasoconstriction was obtained with preparations containing only sufficient propylene glycol to dissolve the steroid. For the creams, it was found that a nearly saturated solution of clobetasol propionate in the aqueous phase (i.e. propylene glycol in water) was required to give optimum release.

Clinical results confirmed these laboratory tests, showing that the amount of propylene glycol in each preparation was critical. In patients with psoriasis there were significantly more preferences for clobetasol propionate 0·05% formulated in a cream base containing 47% propylene glycol than in a base containing 25% ($P<0{\cdot}05$).

Steroid concentration. Early double-blind clinical studies in psoriasis treated without occlusive dressings, showed that, although the number of preferences for a cream containing 0·025% clobetasol propionate exceeded the number for either betamethasone valerate or fluocinonide, the difference did not reach statistical significance. The further clinical investigations were, therefore, carried out using an 0·05% clobetasol propionate cream and ointment.

Comparative clinical evaluation of clobetasol propionate 0·05%

Psoriasis. In patients with psoriasis, there were more preferences for clobetasol propionate than for each of the control steroids, regardless of the base employed or the use or not of polythene occlusion (Fig. 2). Fig. 2 also illustrates the individual comparisons. Preferences for clobetasol propionate ointment when used without polythene occlusion were significant when compared with fluocinonide ($P < 0·01$) and fluclorolone acetonide ($P < 0·05$), but not with betamethasone valerate. When polythene occlusion was employed clobetasol propionate was significantly better than fluocinonide ($P < 0·01$), but was not significantly different from betamethasone valerate.

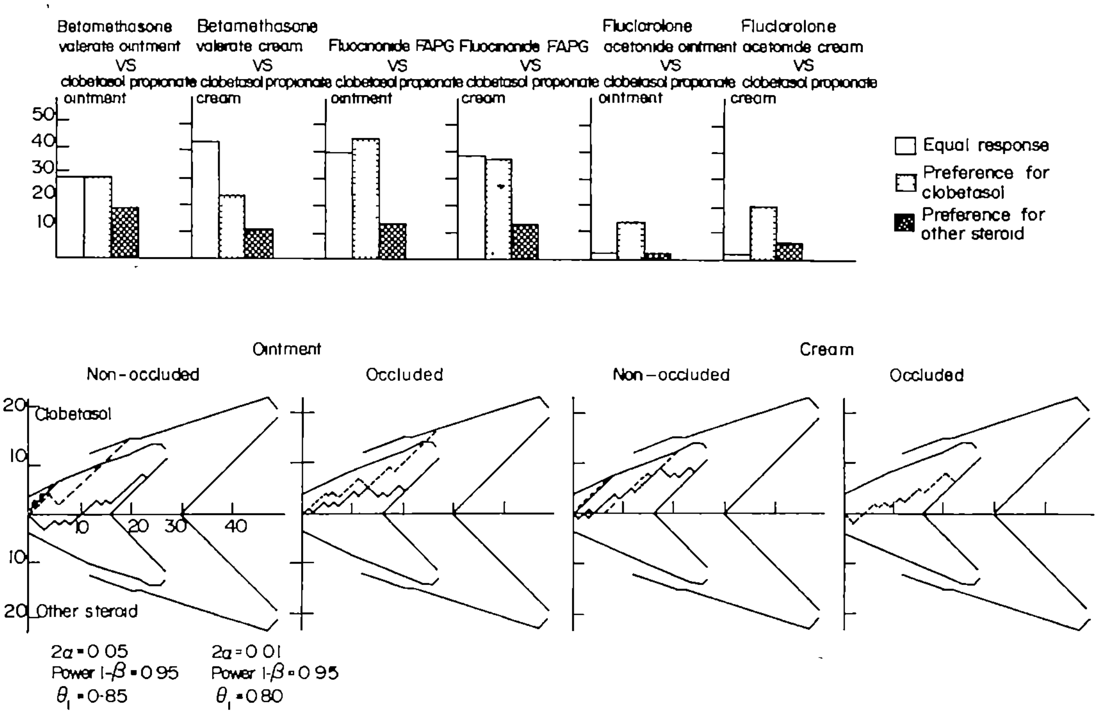


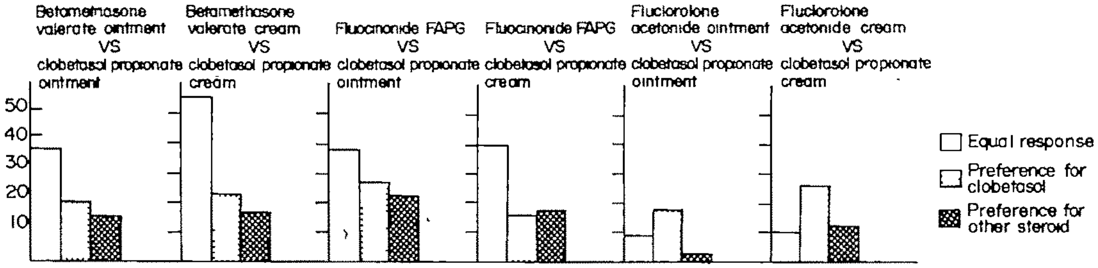
FIGURE 2. Psoriasis—all cases. ——, clobetasol vs betamethasone; – – –, clobetasol vs fluocinonide; ● ● ● ●, clobetasol vs fluclorolone.

Clobetasol propionate cream used without polythene occlusion was superior to fluocinonide and fluclorolone acetonide ($P < 0·05$). However, there was no significant difference between the new steroid cream and betamethasone valerate cream.

Eczema. Similarly, in patients with eczema, there were more preferences for the new steroid than there were for the controls (Fig. 3). However, in this disease category differences were not so marked. Preferences for clobetasol propionate were significantly greater in comparison with fluclorolone acetonide ointment ($P < 0{\cdot}01$) and fluclorolone acetonide cream ($P < 0{\cdot}05$), but not in the other comparisons.

Side effects. Only one of the 1150 patients was not able to complete the treatment because of deterioration equally on the test and the control steroid treated lesions, and he was withdrawn from the trial.

303 patients were asked if they experienced any stinging on application of the cream or ointment.



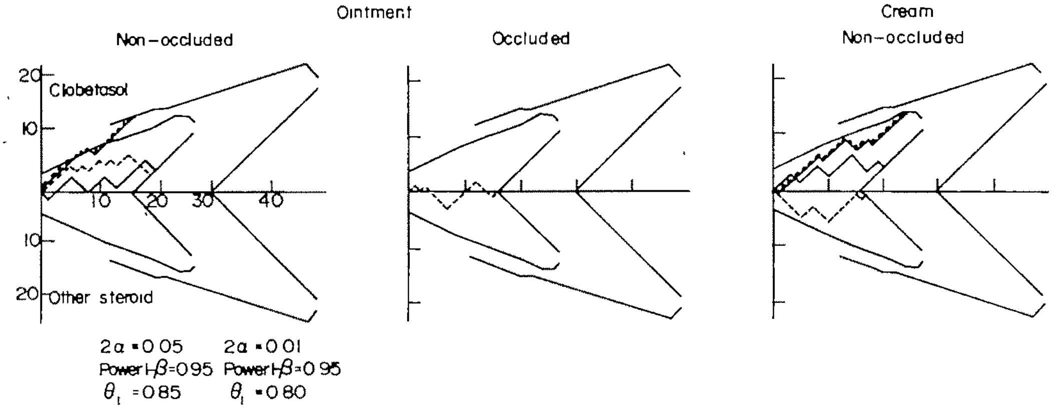


FIGURE 3. Eczema—all cases. ——, clobetasol vs betamethasone; - - -, clobetasol vs fluocinonide; ● ● ● ●, clobetasol vs fluclorolone.

Of these, twenty-seven experienced some sensation with the clobetasol propionate preparations and forty-six with the control preparations. However the effect was usually slight, experienced only on the first application, and in no case did it necessitate interrupting the prescribed regimen.

DISCUSSION

It is recognized that the interpretation of this type of trial has limitations and that it is only after longer term use that the full value of a new topical steroid will emerge. However, short-term assessments have frequently been used in the past and they give a good indication of the relative therapeutic value of compounds (Williams *et al.*, 1964; Munro *et al.*, 1967). Further trials are in progress to assess the response to clobetasol propionate in more detail and in other specific lesions.

Psoriasis is a difficult disease to treat and consequently any differences in potent topical applications show up more clearly in this than in less stubborn diseases. This has been noted by Nicholls (1972) and is apparent in this study, especially when psoriasis was treated without the use of polythene occlusion. Where occlusion was used the differences were not so apparent. Patients are reluctant to use polythene unless it is obvious that their lesions are not going to clear without it; because of this, and its tendency to mask the differences between steroids, we did not study as many occluded as non-occluded cases.

Eczema tends to be less resistant than psoriasis to treatment, so that differences between highly active preparations are not so marked. The majority of preferences were for clobetasol propionate, but statistically significant differences between preparations were not so evident. This was so despite the fact that all the eczema patients had been referred to hospital consultants because of the stubborn nature of their lesions. Patients with eczema were a clinically heterogeneous group ranging from simple acute contact eczemas to chronic lichenified conditions. In a multi-centre study such as this it is difficult, if not impossible, to agree criteria which would enable a more specific categorization of eczema to be undertaken. Were this feasible it is possible that in the more intractable eczemas, more marked differences between the preparations may have emerged.

In this trial no attempt was made to assess the possible side effects of clobetasol propionate. When patients with more than 60% of their body surface affected by disease are treated with topical steroids under total body occlusion, according to the method of James, Munro & Feiwel (1967), all potent steroids cause transient depression of pituitary–adrenal function: clobetasol propionate is not an exception (Munro, personal communication). However, this is no guide to what happens in normal use. Wilson, Williams & Marsh (1973) have shown that betamethasone valerate in out-patient use in reasonable quantities is unlikely to affect pituitary–adrenal function: and this has been confirmed by Munro & Clift (1973). It is not expected that clobetasol propionate will behave differently. The more clinically important steroid side effects of skin atrophy and telangiectasia are not likely to be seen in any trial, since they are the consequence of prolonged misuse of steroids; however, there is no reason to believe that, should this steroid be misused, its propensity to cause such effects would differ from that of any other steroid preparation.

CLINICIANS WHO TOOK PART IN THE TRIAL

United Kingdom

C. F. Allenby	Hitchin
D. A. Birkett	Middlesbrough
A. Bowyer	Torquay
I. W. Caldwell	Southampton
E. S. Emslie	Rhyl
M. Hewitt	Truro
A. R. Kurwa	Birmingham
R. A. Main	Aberdeen
R. Marks	London
J. Overton	Leicester
N. R. Rowell	Leeds
P. D. Samman	London
R. W. B. Scutt	Gosport
D. E. Sharvill	Canterbury
J. R. Simpson	Exeter
C. J. Stevenson	Newcastle
R. S. Wells	London

Sweden

A. Björnberg	Göteborg
J. Bleeker	Lidköping
C. H. Flöden	Karlstad
L. Gip	Sundsvall
L. Hellgren	Göteborg
L. Juhlin	Uppsala
B. Magnusson	Malmö

Finland

A. Lassus	Helsinki

Belgium

J. Van der Meersch	St Niklaas

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British Journal of Dermatology (1974) **90**, 205.

Case Reports

Erythema nodosum with superficial vein involvement

T.J.DELANEY AND BARBARA LEPPARD*

St George's Hospital, London, S.W.1

Accepted for publication 26 June 1973

SUMMARY

A case of erythema nodosum associated with Septrin treatment is presented. During the course of the disease atypical nodules involving superficial veins were observed.

Erythema nodosum was first described by Willan (1808): 'In the Erythema nodosum, many of the red patches are large and rounded. The central parts of them are gradually elevated, and on the 6th or 7th day form hard and painful protuberances which are often taken for imposthumes,† but from the 7th to the 10th they constantly soften and subside without ulceration. On the 8th or 9th day the red colour changes to bluish or livid, and the affected limb appears as if severely bruised. This appearance remains for a week or ten days when the cuticle begins to separate in a scurf.

The Erythema nodosum usually affects the fore parts of the legs'.

Many later authors have confirmed these clinical observations in the typical case (Weinstein, 1969; Hannuksela, 1971), but a number of atypical patterns have been recorded, including erythema nodosum migrans (Bäfverstedt, 1968) and chronic erythema nodosum (Fine & Meltzer, 1969). Various manifestations other than skin lesions may also appear in association with erythema nodosum, of which polyarthritis is perhaps the most common (James, 1961).

We present a case developing unusual superficial lesions during the course of erythema nodosum.

CASE REPORT‡

A 49-year-old woman presented in May 1972 with a 2-day history of painful red nodules on her shins and right elbow, together with joint pain and swelling. Clinically the skin lesions were typical of erythema nodosum. There was some swelling of the wrists and ankles.

Two months before her attendance she had had an episode of frequency and dysuria, for which she was given Septrin (trimethoprim-sulphamethoxazole), three tablets daily for the 4 weeks prior to the development of her rash. She had had no other significant illness. She had received BCG inoculation 2 years earlier, when she had commenced a nursing course.

She was treated symptomatically with analgesics and bed rest. The skin lesions and joint symptoms

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* Present address: St John's Hospital for Diseases of the Skin, London, W.C.2.

† Abscesses.

‡ This patient was shown at the meeting of St John's Hospital Dermatological Society on 29 June 1972.

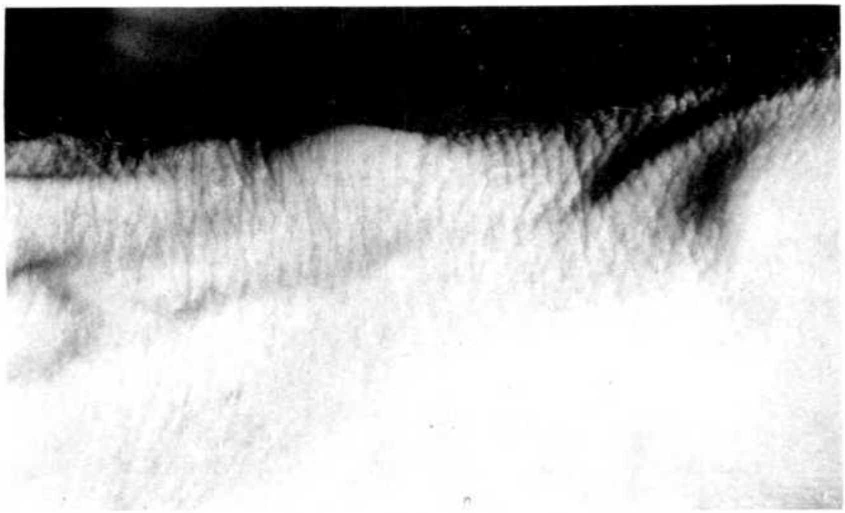

FIGURE 1. Nodule on dorsum of hand along the course of superficial vein.

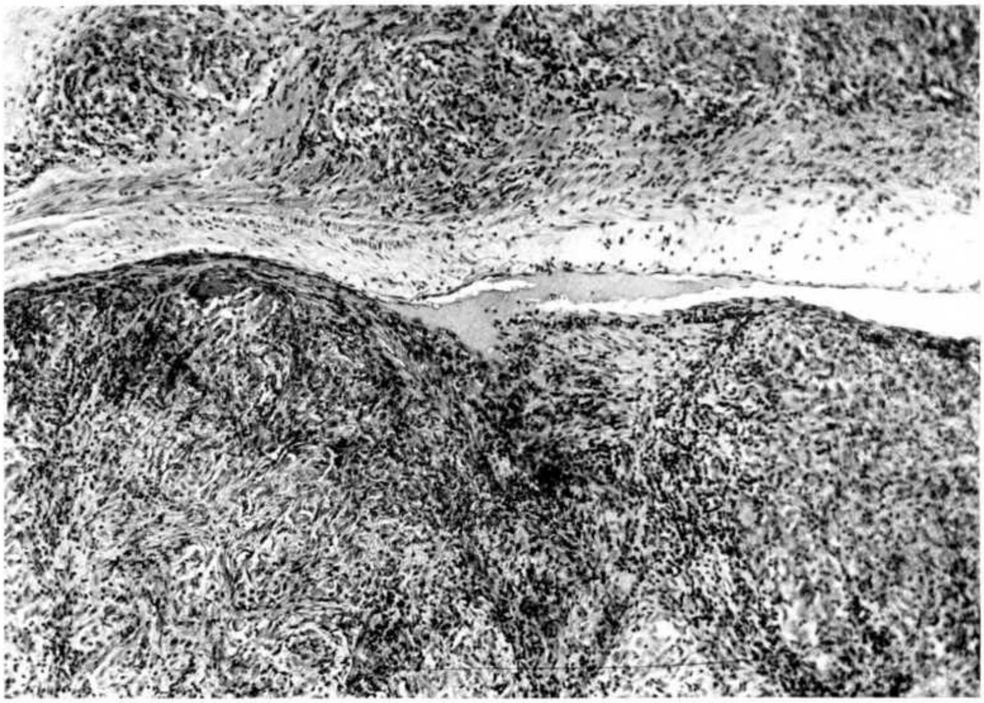

FIGURE 2. Granulomatous infiltrate involving walls of large vein and almost occluding lumen (H & E. × 50).

gradually subsided, the skin nodules going through the usual bruising phase. However, 3 weeks after the initial crop, she developed further typical erythema nodosum lesions and a recurrence of her polyarthralgia. On this occasion she noticed the presence of four very superficial painful nodules situated on the dorsa of her hands, together with a similar nodule on her forehead and one on her lower chest. All of these lesions were firm, mobile, discrete lumps in a superficial subdermal position. The lesions on the dorsa of the hands were obviously intimately connected with the superficial veins (Fig. 1); obstruction to blood flow in the veins by the nodules could be demonstrated.

Excision biopsy was performed on a lesion on the dorsum of the hand, approximately 5 days old, the vein being identified and ligated on both sides of the lesion before excision. Histological examination (Figs. 2 and 3) showed the pathological changes to be within and around the walls of a vein in the subcutaneous tissues. The walls of the vein were infiltrated with a non-caseating granulomatous mass composed mainly of histiocytes and epithelioid cells, but with large numbers of giant cells of both Langhans' and foreign-body types. The lumen of the vein was compressed to a slit, but there was no evidence of thrombosis. The epidermis and superficial dermis were unremarkable.

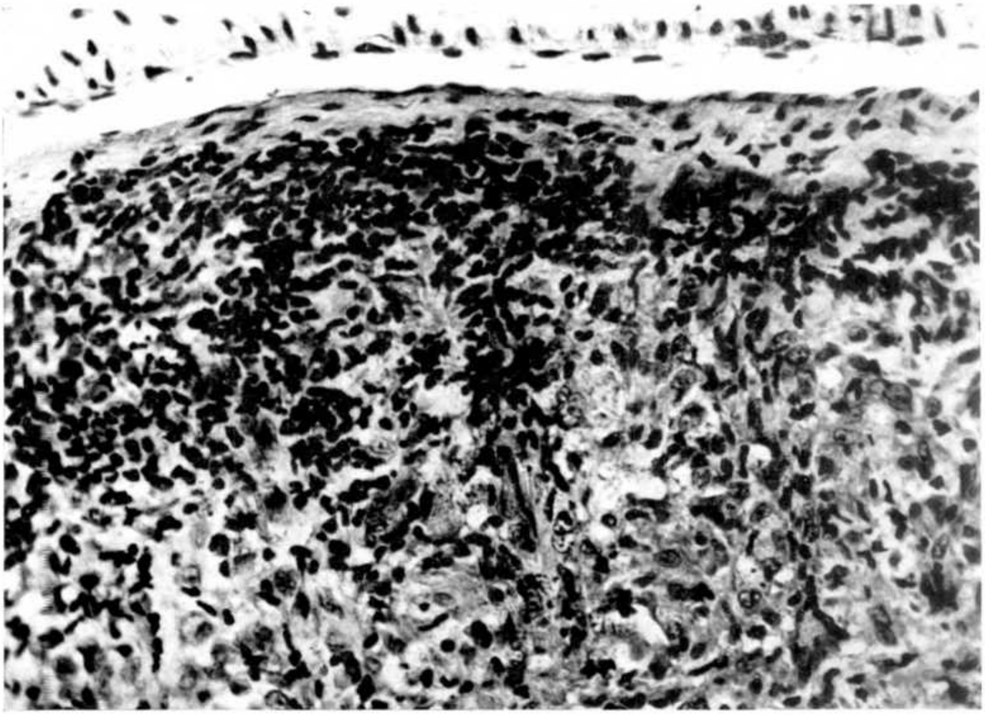

FIGURE 3. View of infiltrate in close proximity to lumen of vein showing lymphocytes and histiocytes (H & E, × 180).

The Mantoux test was positive with 10 TU. Other positive investigations included a raised ESR (80–100 mm/h) and slightly elevated serum α_2 and γ globulins. Negative or normal investigations included: throat swabs, ASO titre, chest X-ray, LE cell and autoantibody investigations, urine analysis with culture and examination for acid fast bacilli.

All signs and symptoms resolved within a further 4 weeks. Blood flow was restored in the involved veins. The ESR returned to normal as did her protein electrophoretic pattern.

One year later she had had no recurrence of her symptoms.

DISCUSSION

It is well recognized that the histology of a typical erythema nodosum lesion shows a granulomatous infiltrate with involvement of the medium sized veins (Löfgren & Wahlgren, 1949; Lever, 1967; Ryan & Wilkinson, 1972). However, not all cases show prominent venous involvement, and the mechanisms of production of lesions are obscure. In this case the infiltrate is similar to that of classical erythema nodosum, but the site and degree of infiltration are atypical.

We feel that the lesions demonstrated by this case may be the 'pure' lesions of erythema nodosum. Because they occurred in an area where there are excellent anastamotic venous channels, secondary anoxic change or haemorrhage did not occur. As the lesions were very superficial, accurate biopsy was possible.

Although we have not challenged the patient with Septrin or its component drugs, we suggest that this case of erythema nodosum is likely to be secondary to the sulphonamide component of Septrin, and that the superficial nodules are an atypical clinical manifestation of erythema nodosum.

ACKNOWLEDGMENT

We would like to thank Dr K.V.Sanderson for permission and encouragement to publish this case report.

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Erythema nodosum and oral contraceptives

L.G.DARLINGTON

St Stephen's Hospital, Fulham Road, London, S.W.10

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SUMMARY

Erythema nodosum may be related to a variety of diseases or possibly to drug therapy. Oral contraceptives have only rarely been implicated in the literature, but a number of cases have been reported to the Committee on Safety of Medicines in which erythema nodosum; appears to have been related to contraceptives, in fact, they are the drugs most commonly reported to this Committee as associated with the disorder. A detailed case report is presented in which erythema nodosum is thought to have been precipitated by an oral contraceptive.

Whilst several groups of drugs have been implicated in its aetiology, a causative relationship between a drug and erythema nodosum is often questionable. Oral contraceptives and erythema nodosum have been linked by several authors (Holcomb, 1965; Matz, 1967; Baden & Holcomb, 1968; Savel, Madison & Meeker, 1970). Savel *et al.* (1970) in fact described not only erythema nodosum, but also erythema multiforme in their two patients taking oral contraceptives.

CASE REPORT

The patient was a 25-year-old white woman doctor, who began taking oral contraceptives on 17 April 1971. The drug combination taken was Minilyn, i.e. lynestrenol and ethinyloestradiol, a product which has not previously been implicated as causing erythema nodosum.

On the same day that the oral contraceptive was begun, there was fever and generalized malaise, which was proved, a fortnight later, to be due to infectious mononucleosis, as shown by a positive Paul Bunnell test and atypical mononuclear cells. There was rapid improvement and the blood picture and ESR reverted to normal.

Three months later during the third full cycle of the contraceptive, the patient noticed the sudden onset of tiredness, severe aching in the legs, cervical and inguinal lymphadenopathy, and tender, red nodules on both legs, characteristic of erythema nodosum. There was slight improvement during the week off the contraceptive and she was advised to stop taking it. On her own initiative she restarted, and during this cycle, 4 months after starting oral contraceptive therapy, there was severe malaise, accompanied by headache and pains in the ankles, elbows, wrists and right knee. Symptoms became even more severe when she changed in mid-cycle to an alternative, low-dose oral contraceptive, Norinyl-1 (norethisterone and mestranol). At this time, the red, shiny lesions became more numerous on both legs: they were about 1 cm × 1 cm in size, confluent and very tender. At this stage the patient developed nocturia. Symptoms returned to a tolerable level when she reverted to her original

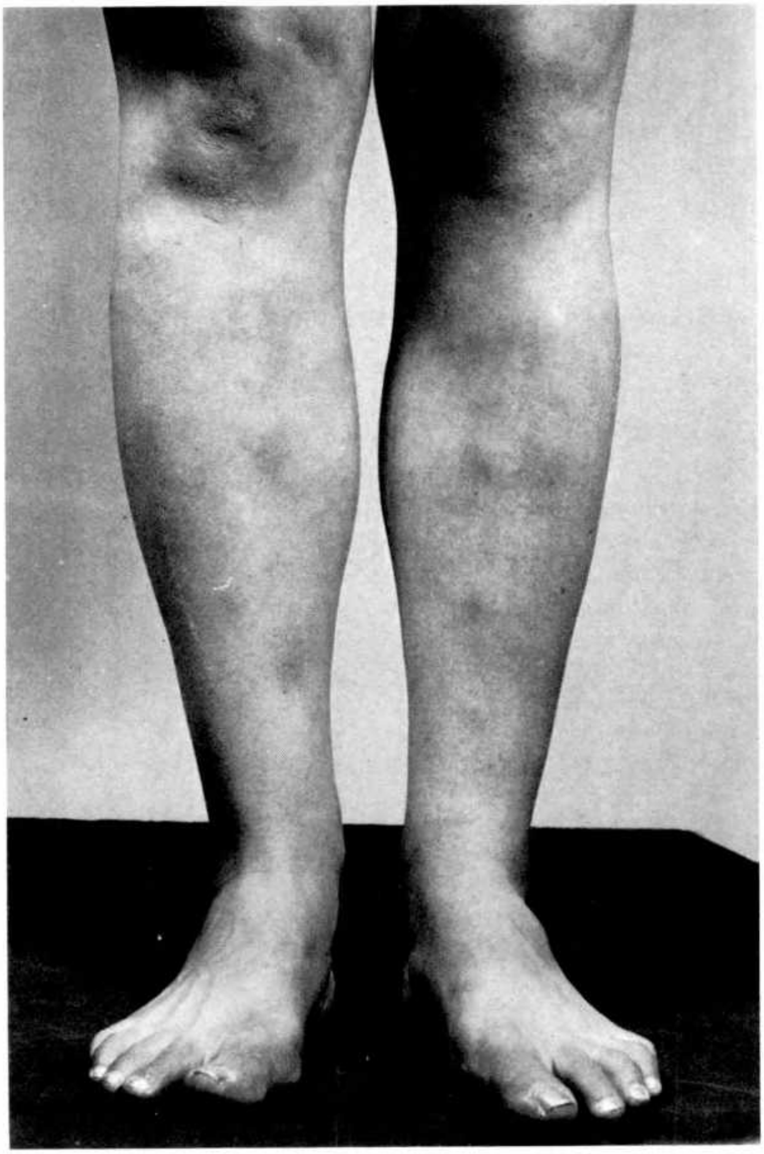

FIGURE 1. Erythema nodosum in patient taking oral contraceptives.

oral contraceptive, and she remained actively ambulant. After a few more days, a nodule had spread to the left thigh and there was pitting oedema up to both knees. The oral contraceptive was now stopped. Arthralgia, weakness, malaise and oedema continued for several weeks, but the nodules faded slowly, the lymph nodes returned to their usual size, and normal health was regained.

The past history revealed severe reactions to insect bites, mild wheezing associated with emotional stress, and rigors and malaise for 1 day, 3 years previously, when taking fenfluramine to assist weight reduction. She was taking no other drugs at the time of the onset or during the course of the erythema nodosum, and the only drugs taken in the previous 4 months had been soluble aspirin, in small doses during infectious mononucleosis, and iodochlorhydroxyquinoline (Enterovioform) for 1 week when on holiday in June.

INVESTIGATIONS

The ESR was 4 mm/1 h and blood counts were consistently normal at a time when both the skin lesions and the malaise were prominent. Blood urea, antistreptolysin titre, antinuclear factor, sheep cell agglutination and latex tests showed no abnormality, and a search for LE cells was negative on three occasions. The Paul Bunnell test was found to have reverted to negative 14 months after the onset of the glandular fever. An attempt was made to exclude *Pasteurella pseudotuberculosis* infection by a Widal test, which was negative: this does not absolutely exclude this infection. A throat swab showed no pathogens and especially no beta-haemolytic streptococci. A Mantoux test 1 year previously had been positive at a strength of 1 in 1000 and was still positive at this strength. Four early morning specimens of urine showed no evidence of tuberculosis or other abnormality.

Although a normal chest X-ray does not exclude sarcoidosis, no evidence of the condition was found on postero-anterior and lateral chest films, when symptoms of erythema nodosum first developed. In a further attempt to exclude a diagnosis of sarcoidosis, serum calcium, serum proteins (total protein, albumin, gammaglobulins and an electrophoretic strip), and a latex fixation test were performed and were all normal.

DISCUSSION

Oral contraceptives were implicated as a cause of erythema nodosum by Holcomb (1965), whose patient developed erythema nodosum on a preparation containing norethynodrel and ethinyl oestradiol; she was later able to tolerate a product containing ethynodiol diacetate and mestranol (Table 1).

The patient described by Baden & Holcomb (1968) developed erythema nodosum whilst on an oral contraceptive combination containing norethindrone and mestranol, but tolerated well a contraceptive containing ethinyl oestradiol and dimethisterone.

Baden & Holcomb (1968) noted that norethindrone and norethynodrel, the first progestational agents given in their own case and in that of Holcomb (1965), were almost identical in chemical structure, except for the position of the double bond. They suggested that these progestational compounds could be the cause of the erythema nodosum, but they could not establish this with certainty.

In contrast, however, in both cases recorded by Savel *et al.* (1970) the addition of mestranol to *in vitro* cultures of the patients' peripheral blood lymphocytes resulted in stimulation of lymphoblast formation, as measured by the uptake of tritiated thymidine. The test was negative for progestin. They suggested, therefore, that the erythema nodosum in these cases could represent hypersensitivity to oestrogenic components of oral contraceptives. With a reaction such as erythema nodosum, which may be caused by a variety of agents not always identifiable in the individual case, it is difficult to establish a causal relationship with any one drug, especially when for many months there have been no apparent side effects to the drug. Similar difficulties arose in the case recorded above; the drug had been taken for 3 months before the erythema nodosum appeared. A latency of 8 months was noted by Holcomb (1965), and 15 and 21 months by Savel *et al.* (1970).

There is, moreover, room for suspicion that drugs other than oral contraceptives may have been involved in all of the previously recorded cases, except one of two described by Savel *et al.* (1970). Six weeks before the development of erythema nodosum, Holcomb's (1965) patient had been given tetracycline for an upper respiratory tract infection, while Baden & Holcomb's (1968) patient had

TABLE 1. Contraceptive medication

Patient	First drug	Second drug
Holcomb (1965)	Norethynodrel and ethinyl oestradiol	Ethynodiol diacetate and mestranol
Baden & Holcomb (1968)	Norethindrone and mestranol	Ethinyl oestradiol and dimethisterone
Savel *et al.* (1970)		
Patient 1	Norethindrone and mestranol	—
Patient 2	Ethynodiol diacetate and mestranol	—

Generic and trade names of drugs

Ethynodiol diacetate and mestranol	Ovulen, Demulen
Norethindrone and mestranol	Norinyl-1 or 2, Ortho-Novin
Ethinyl oestradiol and dimesthisterone	Oracon, Tova
Lynestrenol and ethinyl oestradiol	Minilyn

received penicillin G, ampicillin and a sulphonamide, all of which were stopped only a few days before the erythema nodosum appeared. The first patient described by Savel *et al.* (1970) had been given aspirin, a mixture of phenylpropanolamine and chlorpheniramine (Contac), and chlorpheniramine and phenylephrine mixture, and had also taken hydrochlorothiazide (Hydrodiuril) daily for 1½ years for mild hypertension. All drugs were discontinued after consultation and none was restarted, so the causal agent was not detected. In the present case, the only drug taken within 2 months of the erythema nodosum was Enterovioform, which has never been reported in association with this side-effect, and which had previously been taken uneventfully by this patient.

CONCLUSIONS

It is thus impossible, in the small number of recorded cases of erythema nodosum which are believed to be secondary to the use of oral contraceptives, to be sure that the drugs taken were really the cause of the reaction.

However, the present case and the earlier reports suggest that oral contraceptives may possibly cause erythema nodosum in their own right or, perhaps more probably, that they may so influence the hormonal milieu in some way at present not understood that erythema nodosum is more likely to occur from other causes.

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Porphyria cutanea tarda in a 2-year-old girl

A.KANSKY

Dermatoloska Klinika, Ljubljana, Zaloska, C.2., Jugoslavia

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SUMMARY

The paucity of published reports suggests that porphyria cutanea tarda is very rare in children. A case is reported in which the clinical and laboratory data suggest that the disease started at the age of 2 years.

According to Berman & Bielicky (1956), Ippen (1959), Canivet & Fallot (1959), and others, the average age at the onset of porphyria cutanea tarda (PCT) is about 50 years; our own experience is similar (Kansky, 1967). There are few reports of PCT in children, e.g. Schmid, Schwartz & Watson (1954) of a 5-year-old, Gajdos *et al.* (1963) of a 7-year-old, and Welland and Carlsen (1969) of an 8-year-old. During the last 2 years we had under observation a case of PCT which appeared in a 2-year-old girl.

CASE HISTORY

The girl, born 20 December 1968, was admitted to our department for the first time on 2 November 1971. She was born 27 days premature at a birth weight of 2·6 kg, and measured 46 cm. At the age of 1 year the girl had measles; she suffered repeatedly from sore throat and left sided otitis. At the age of 1½ years, during the early summer, small vesicles appeared for the first time on her face and on the backs of the hands. The vesicles were followed by crusts and scars. During the winter the lesions disappeared, only to recur next summer. The diagnosis of porphyria was made at the Department of Dermatology in Zagreb; later the parents consulted us for further evaluation.

Family history

Both parents were healthy except that the father had vitiligo. In both, porphyrins in urine and blood were normal. Both parents originated from the same small village but there was no consanguinity. The father's parents and siblings were healthy, though two of the latter had gastric ulcer. The mother of the propositus was an only child and her parents were healthy. The patient's younger brother was healthy.

Clinical findings

Physical examination revealed a normally developed and mentally alert girl. Her skin was pale. There were a few decayed teeth. On her nose, upper lip and the pinna there were isolated crusts

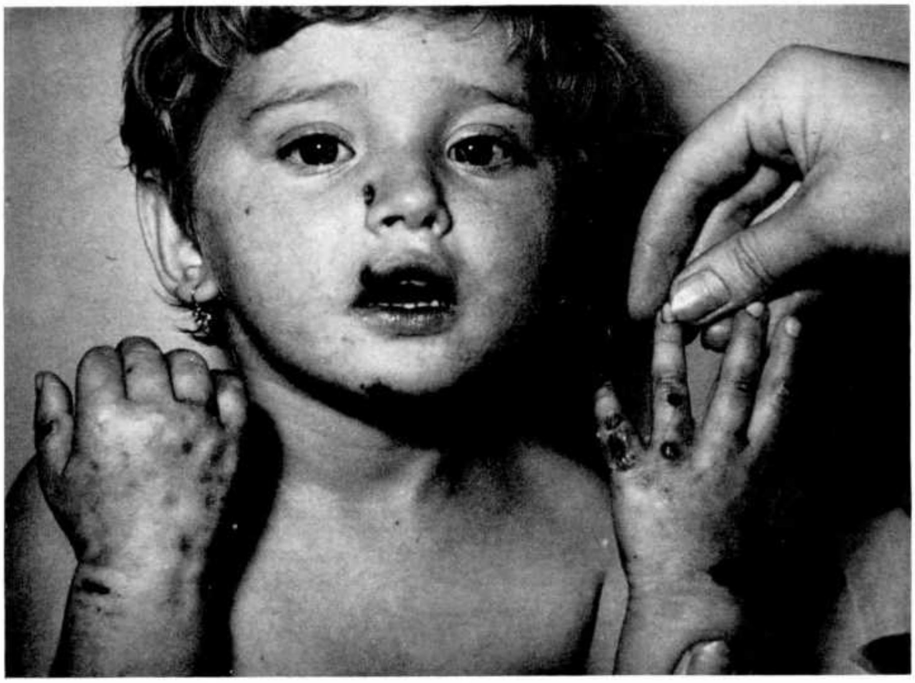

FIGURE 1. To show vesicles and crusts on exposed skin.

TABLE 1. Summary of laboratory findings

Investigation	Date			
	2.11.71	24.4.72	27.6.72	25.10.72
Urine				
CP μg/1000 ml	200	123	52	160
UP μg/1000 ml	6360	2370	1330	1790
PBG mg/100 ml	0·19	0·1	0·40	0·20
ALA mg/100 ml	0·85	0·3	0·52	0·30
Red blood cells				
CP μg/100 ml	1·0	3·9		
PP μg/100 ml	6·6	20·0		
Serum transaminases				
GOT units	95		62	80
GPT units	98		42	43

CP, coproporphyrin; UP, uroporphyrin; PP, protoporphyrin; PBG, porphobilinogen; ALA, delta aminolaevulinic acid; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvate transaminase.

and a number were present also on the backs of the hands. These areas also showed a number of small pink, depigmented or slightly hyperpigmented scars (Fig. 1). The finger nails had no lunulae.

Laboratory data. Haemoglobin 11·4 g/100 ml, red blood cells 3×10^6/mm^3, leukocytes 10,500/mm^3, glutamic sedimentation rate 45 mm in 1 h. Serum: glutamic oxaloacetic transaminase 95 Wroblewski units (normally up to 40), glutamic pyruvate transaminase 98 Wroblewski units (normal up to 40), iron 40 μg/100 ml, unsaturated iron binding capacity 345 μg/100 ml; serum protein electrophoresis showed lowered albumin and increased α_2 and γ globulin, total protein 7·7 g/100 ml. Urine: porphobilinogen 0·19 mg and delta-aminolaevulinic acid 0·85 mg/100 ml; coproporphyrin 200 μg and uroporphyrin 6360 μg/1000 ml. Red blood cells: coproporphyrin 1 μg and protoporphyrin 6·6 μg/100 ml packed cells.* Routine urine analysis, thymol turbidity, serum cholesterol and fluorescence microscopy of the red blood cells were normal. Under Wood's light the teeth showed no red fluorescence.

Follow-up

During the next 6 months a few new crusted lesions developed on the face and dorsal aspects of the hands. Urinary coproporphyrin isomer assay (Dr Sydney Smith, Cardiff): Isomer III 57%, Isomer I 43%. The results of repeat porphyrin analyses in the urine and blood are shown in Table 1.

COMMENT

The biochemical findings were compatible with a diagnosis of PCT in this child. The urinary uroporphyrin was markedly raised, the coproporphyrin only slightly raised. In addition, the urinary coproporphyrin isomer assay was in keeping with PCT. There appeared to be some underlying hepatic abnormality judging from the raised serum SGOT and SGPT but unfortunately we were unable to obtain a liver biopsy. A further interesting finding was the increase in urinary δ-aminolaevulinic acid.

We could obtain no evidence of contact with any hepatotoxic compounds.

ACKNOWLEDGMENTS

I am grateful to Dr Sydney Smith and Mr R.V.Belcher of the Department of Medicine, University Hospital of Wales, for performing the isomer assay.

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* In our laboratory porphobilinogen and aminolaevulinic acid are assayed according to Mauzerall & Granick (1956), coproporphyrin and uroporphyrin in urine according to Askevold (1951), coproporphyrin and protoporphyrin in red blood cells according to Rimington *et al.* (1963). Porphobilinogen up to 0·30 mg, aminolaevulinic acid up to 0·54 mg/100 ml of urine, coproporphyrin up to 104 μg and uroporphyrin up to 10 μg/1000 ml urine. In red blood cells coproporphyrin up to 7·6 μg and protoporphyrin up to 18·8 μg/100 ml of packed cells.

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Multiple extra-medullary plasmacytomas of the skin

CASE REPORT WITH A NOTE ON PROGNOSIS

L.STANKLER AND J.F.DAVIDSON

Aberdeen Royal Infirmary, Aberdeen, and Glasgow Royal Infirmary, Glasgow

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SUMMARY

Multiple plasma cell tumours of the skin may, on rare occasions, precede clinical, radiological and laboratory evidence of systemic myelomatosis. A man presented with several plasma cell tumours of the skin but with no detectable evidence of the disseminated myelomatosis to which he succumbed 16 months later. Multiple cutaneous extra-medullary plasmacytomas should be regarded as a cutaneous expression of multiple myelomatosis.

Extra-medullary plasmacytomas are uncommon (Innes & Newell, 1961). Those involving the skin are rare (Bluefarb, 1955) and, in the absence of evidence of underlying systemic involvement, very rare (Hellwig, 1943). Although multiple cutaneous extra-medullary plasmacytomas have been described, the case presented, as far as can be ascertained from the literature, appears to be unique, in that it is the only one without evidence of associated systemic involvement at the time of diagnosis of the cutaneous lesions.

CASE REPORT

A man aged 43 presented in April 1966 with a 2 months' history of painless enlarging nodules. There was no relevant past or family history. On examination, six tumours were noted, all non-tender, and measured 1–3 cm in diameter. One over the right biceps had the colour of, but was not attached to, the skin. Of three lesions in the left axilla, two were skin-coloured and mobile, and one was blue, with overlying telangiectases and was attached to skin (Fig. 1). The other two lesions, one above the left clavicle and the other in the groin, were mobile and skin-coloured. General examination was otherwise normal. The ESR was 45 mm/1 h (Westergren), but other haematological investigations were normal. Marrow granule smears did not show any evidence of myeloma, skeletal survey was negative, serum electrophoresis did not show a paraprotein band, and immunoelectrophoresis (Dr Rowe, Department of Experimental Pathology, Birmingham) was normal (Table 1). The lesion over the right biceps, and the telangiectatic lesion were excised and showed similar histological features; each lesion lay deep to the dermal appendages and consisted of nodules of very cellular areas, surrounded by fibrous tissue and non-specific granulation tissue. The cellular areas were formed by sheets of cells with a scanty

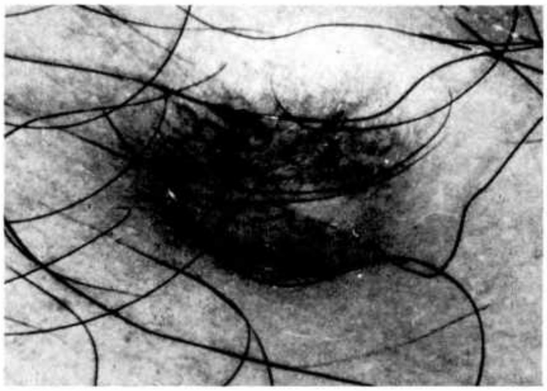

FIGURE 1. Clinical appearance of lesion in left axilla.

reticulin framework. Many of the cells were undifferentiated and showed numerous mitotic figures. Others were typical plasma cells, and of these there were binucleate forms, some showing mitotic activity (Fig. 2). The patient was considered to be suffering from multiple cutaneous extra-medullary plasmacytomas.

The superficial nodules were treated by local radiotherapy; a field of 12 cm × 12 cm was directed towards the left axilla, and an 8 cm × 6 cm field to the left supra-clavicular fossa. An incident skin dose of 3500 rads was delivered to each area over 3 weeks, and the nodules resolved rapidly. The patient's general health remained good, and the ESR returned to normal. Seven months later, his general condition showed some deterioration, and the ESR began to rise. In December 1966, he developed toothache, numbness of the lower lip and chin, and clumsiness of the tongue. X-rays of skull and jaws were normal, but serum protein electrophoresis showed a paraprotein band in the gammaglobulin region; Bence-Jones' protein was found in the urine; the ESR was 48 mm/1 h. Marrow smears were diagnostic of myeloma. In January 1967, a tender lymph node was palpable above the right clavicle, the ESR was 100 mm, and immunoelectrophoresis showed a rise in IgA (Table 1).

Phenylalanine mustard therapy was instituted in a dose of 6 mg/day for 7 days, and 2 mg daily

TABLE 1. Immunoglobulin levels at various stages throughout patient's illness. Increase in IgA-type L myeloma protein

	Date		
	19.7.66	23.1.67	20.6.67
IgA (% standard) (40–200)	140	268	900
IgG (mg/100 ml) (600–1600)	1080	750	780
IgM (% standard) (40–300)	50	123	58
IgD (% standard) (0–100)	—	24	5

thereafter. The ESR remained elevated, but there was symptomatic improvement. The supra-clavicular node resolved, and, after 2 weeks of therapy, Bence-Jones' protein was not found in the urine.

In March 1967, phenylalanine mustard had to be withdrawn for several weeks because of leukopenia.

In April 1967, the patient's general condition deteriorated rapidly, and lytic lesions were seen in X-rays of the skull and mandible. A paraprotein band was again present on serum protein electrophoresis, and Bence-Jones' protein was again found in his urine. Despite further phenylalanine mustard therapy, no further clinical or biochemical improvement was achieved. In June 1967, immunoelectrophoresis revealed a further rise in his IgA level (Table 1). Further radiotherapy was given to swellings in the scalp, trunk and lumbar spine, but his condition deteriorated and he died at home in August 1967, some 16 months after his first visit to hospital.

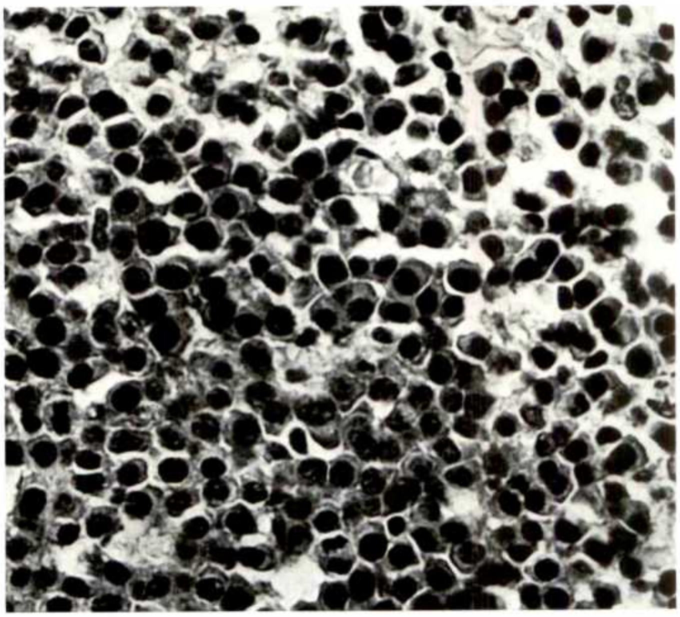

FIGURE 2. Histological features of lesion in left axilla (H & E, × 400).

DISCUSSION

Patients with multiple myeloma may present with non-specific skin lesions, such as figurate erythema (Summerly, 1964), amyloid lesions (Cohen, 1967), pyoderma gangrenosum-like lesions (Jablonska, Stachow & Dabrowski, 1967) and cryoglobulinaemia (Cowan, 1961).

Solitary and multiple extra-medullary plasmacytomas of the skin may occur in association with soft tissue plasmacytomas involving other organs (Hayes, Bennett & Heck, 1952; Switzer, Moseley & Cannon, 1950), or may form part of the picture of multiple myeloma (Zanca *et al.*, 1964; Beevers, 1972; Haribhakti, 1966; Wuepper & MacKenzie, 1969). Multiple skin lesions may also be an expression of plasma cell leukaemia (Piney & Riach, 1931; Newman *et al.*, 1952). Solitary lesions with no systemic involvement have been reported in only nine patients (LaPerriere, Wolf & Gellin, 1973). The patient described by Pianzola, Casteletto & Drut (1972) had a solitary plasmacytoma of the left inframammary region. Skeletal survey was normal as was marrow cytology, but the patient's ESR was 96 mm/1 h and his immunoglobulin pattern showed a monoclonal gammopathy of the IgA type. As far as can be ascertained from the literature, only one previous case of multiple cutaneous extra-medullary plasmacytomas without any underlying abnormality has been reported (Fayolle, 1963), but unfortunately that case was poorly documented and associated multiple myeloma cannot be excluded.

Plasma cells may predominate in ulceration of the mouth, eosinophilic granuloma (Caldwell, 1962) and plasma cell balanitis (Zoon, 1952), conditions which should be considered in the differential diagnosis.

The prognosis in patients presenting with solitary or multiple extra-medullary plasmacytomas, who have myeloma or leukaemia, is that of the underlying disease. Patients presenting with cutaneous lesions as part of soft tissue lesions of various organs invariably develop myeloma (Switzer *et al.*, 1950). The prognosis in patients with solitary cutaneous extra-medullary plasmacytomas, who have no evidence of underlying involvement, is variable. The patient described by LaPerriere *et al.* (1973) had a solitary cutaneous plasmacytoma for 20 years. In Krutsay's (1959) patient there was no recurrence 1 year after local excision. The patient reported by Mikhail, Spindler & Kelly (1970) had metastatic spread to a regional lymph node from a solitary plasmacytoma cutis. After excision of both lesions there has not been any evidence of multiple myeloma. In the patient described by Johnson & Taylor (1970). following the excision of a solitary skin lesion there was spread to the regional lymph node with extension to the peritoneal cavity and eventual involvement of liver, pancreas and thyroid. The bone marrow was not affected. There is still argument as to whether multiple plasmacytomas result from metastatic spread or whether they originate from multiple primary foci. Johnson & Taylor (1970) believed that the lesions in their patient resulted from metastatic spread. The widespread indiscriminate cutaneous and systemic involvement in the present patient is more in keeping with an origin in multiple primary foci.

According to Stout & Frericks (1949), the term solitary extra-medullary plasmacytoma should be applied only to tumours in which disseminated myelomatosis can be excluded by radiological, haematological, chemical and morphological investigation. The present patient appears to be the only one reported with multiple cutaneous lesions but without evidence of systemic involvement at the onset and, until further cases are described, patients with multiple cutaneous extra-medullary plasmacytomas should be regarded as suffering from multiple myeloma.

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Comment

THE EPIDERMIS AND CYCLIC AMP

A hormone induces a rapid and profound change in the metabolic balance of its target organ. Early studies emphasized the extraordinarily low concentrations in which many hormones are effective. The fact that catecholamines and polypeptides are frequently unable to penetrate the surface of cells made this seem all the more remarkable. The mechanism of their action, which for many years remained a central problem of molecular biology, was finally resolved by the discovery of adenosine 3′,5′-monophosphate (cyclic AMP) in 1958 by Sutherland & Rall. It subsequently became apparent that cyclic AMP was the intracellular second messenger for those hormones that by themselves could not penetrate inside the cell.

An overall but abbreviated sequence of events is shown in Fig. 1. When activated by cyclic AMP, a cyclic AMP dependent protein kinase may phosphorylate many different proteins, both enzymatic and structural. In some cases this results in their activation, whereas in others the protein is converted to a biologically inactive form. Thus, co-ordinate control over several different pathways is accomplished. In addition, the amplification obtained by linking enzymic reactions in cascade is considerable; one may visualize how a cell could respond to a very few molecules of the external hormone (the 'first messenger'). The most comprehensive review of the general literature is the monograph of Robison, Butcher & Sutherland (1971), which includes a bibliography of over 1500 references to cyclic AMP. It is notable but not surprising that this literature deals with almost every mammalian tissue except epidermis.

Does the 'second messenger' system in fact operate in mammalian epidermis? Adenylate cyclase activity was first described in homogenates of whole guinea-pig skin (Mier & Urselmann, 1970a), but no hormonal stimulation could be demonstrated. Using a particulate fraction isolated from epidermis, Duell *et al.* (1971) and Marks & Rebien (1972a) were able to show some degree of stimulation by catecholamines, although this was still very small compared with other tissues. Intact epidermal cells, on the other hand, respond vigorously; for example, Powell, Duell & Voorhees (1971) found a threefold increase in the cyclic AMP content of rat epidermis following exposure to isoproterenol, and Brønstad, Elgjo and Øye (1971) reported increases of more than ten-fold in the synthesis of cyclic AMP by whole hamster skin in response to adrenalin. Thus, it is now clear that this part, at least, of the system is functional.

The other two enzymes shown in Fig. 1 have now also been described in mammalian skin. The enzyme responsible for lowering cellular levels of cyclic AMP after hormonal stimulation has ceased, cyclic AMP phosphodiesterase, was examined in whole mouse skin (Mier & Urselmann, 1972) and found to have a K_m of 9×10^{-5} M and was inhibited by methyl xanthines. The high K_m cyclic AMP phosphodiesterase has been measured in both involved and uninvolved psoriasis epithelium and appears to be unaltered (Voorhees *et al.*, 1973a). The low K_m activity might be increased in the lesion in comparison to the uninvolved areas (Voorhees *et al.*, 1974a). Cyclic AMP dependent protein kinase has been investigated in whole human skin (Kumar, Tao & Solomon, 1971), in whole mouse skin (Mier & van den Hurk, 1972) and in human epidermis (Voorhees *et al.*, 1974b); the cutaneous enzyme seems very similar to those from other tissues. Although certain of these results pertain to whole skin, the preponderance of epidermal cells in such preparations and the demonstration of all the components of the cyclic AMP system in human epidermis (Voorhees *et al.*, 1974a) makes it

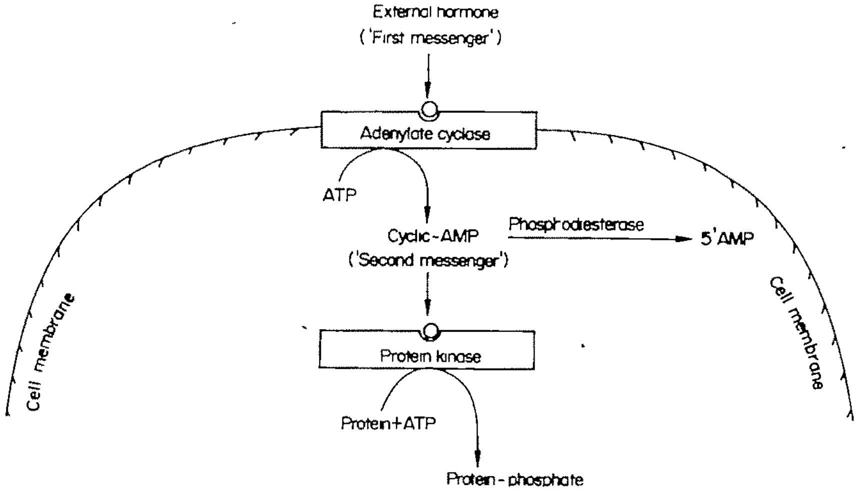

FIGURE 1. The 'cyclic AMP cascade'. Both enzymatic and structural proteins may be phosphorylated by the cyclic AMP-dependent protein kinase.

reasonable to conclude that epidermis is indeed equipped with a complete 'second messenger' system.

But what 'message' does this system carry? The activity of many internal tissues must be linked to the minute-to-minute needs of the body as a whole; since epidermis (as far as we know) does not secrete metabolites vital to other organs, it is more difficult to understand the need for such continuous hormonal supervision. What epidermis does provide, however, is a highly specialized barrier between the 'inside' and the 'outside'. An important parameter, therefore, is its thickness; one must presume the existence of a homeostatic device to balance the proliferative rate of the basal cells with the rate of desquamation from the surface. This homeostasis has been studied extensively by Bullough, who introduced the now familiar concept of the epidermal chalone (Bullough, 1962). In the course of this work it was established that catecholamines are powerful inhibitors of epidermal cell division.

Voorhees & Duell (1971) proposed that cyclic AMP mediates this catecholamine induced inhibition of proliferation. This hypothesis not only assigns a physiological function to the 'second messenger' in epidermis, but also explains (via the activation of glycogen synthetase and the inactivation of glycogen phosphorylase) (Fig. 2) why an accumulation of glycogen generally accompanies enhanced epidermal proliferation. In essence, the Voorhees–Duell hypothesis suggests that cyclic AMP is a major regulator (not necessarily the only one) of epidermal thickness and function. However, which hormone, if any, modulates intraepidermal cyclic AMP levels is unknown. The experimental support for this hypothesis is now considerable, and includes the following observations:

(1) *In vitro* studies have shown that cyclic AMP directly inhibits epidermal cell division in a dose-dependent manner (Marks & Rebien, 1972b; Voorhees, Duell & Kelsey, 1972). In fact, the four criteria of Robison *et al.* (1971) of a cyclic AMP mediated event (inhibition of epidermal cell division) have been met (Voorhees *et al.*, 1973a).

(2) In rapidly dividing epithelium, such as the psoriatic plaque, the level of cyclic AMP *in vivo* is decreased (Voorhees *et al.*, 1972).

(3) The degree of activation of phosphorylase (Mier & van den Hurk, 1974) has been reported as lower than normal in the psoriatic plaque.

(4) Wright *et al.* (1973) claim to have found a reduced rate of cyclic AMP synthesis in the psoriatic

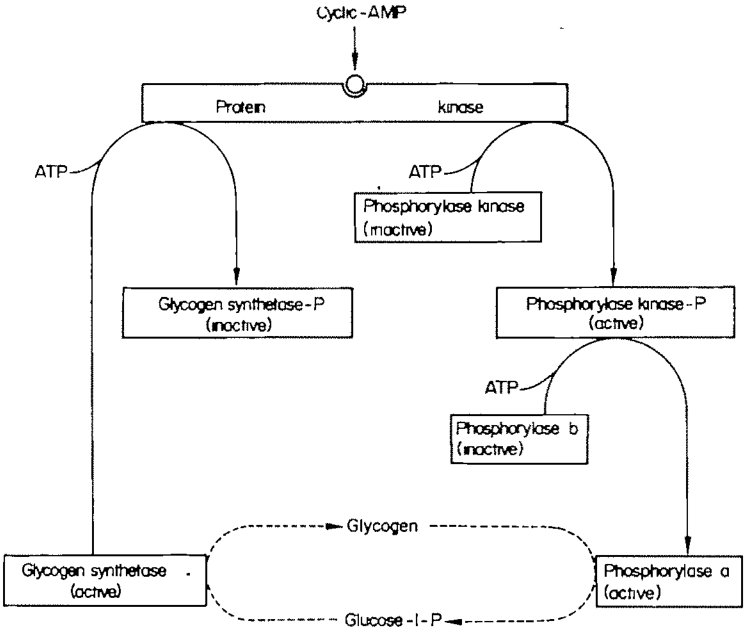
Cyclic-AMP
Protein kinase
ATP
ATP
Phosphorylase kinase (inactive)
Glycogen synthetase-P (inactive)
Phosphorylase kinase-P (active)
ATP
Phosphorylase b (inactive)
Glycogen
Glycogen synthetase (active)
Phosphorylase a (active)
Glucose-1-P

FIGURE 2. Control of glycogen metabolism via the activation of protein kinase. Most of the components of this pathway have now been demonstrated in epidermis.

plaque. This may be the case but requires further work and confirmation, as discussed by Voorhees *et al.* (1973a).

Can the pathogenesis of any of the familiar dermatoses be related to a defective 'second messenger' system? So far two hypothesis have been offered. The earlier of these was the attempt by Szentivanyi (1968) to describe the clinical manifestations of atopy in such terms. Despite considerable circumstantial evidence in support of this idea, direct measurements of the three enzymes concerned (Fig. 1) in four to six patients have failed to reveal any abnormality (Mier & Urselmann, 1970b; Holla *et al.*, 1972; Mier *et al.*, 1972). Although the enzyme activities are apparently normal *in vitro*, any final conclusions must await study of factors which may interfere with the function of these enzymes *in vivo* and the study of the adenylate cyclase receptors at the cell surface.

The more recent, and more promising, line of thought relates to psoriasis. The original suggestion of a defective cyclic AMP cascade in this disease was made by Voorhees & Duell (1971). As we have already pointed out, subsequent reports seem to support this notion that both the high proliferative rate and the accumulation of glycogen in the psoriatic lesion are due to a co-ordinate misregulation occasioned by reduced cyclic AMP levels. This provides an important new link in the pathogenic chain, but in no sense tells us the 'primary cause' of the disease. It would, of course, be tempting to equate the familial predisposition to psoriasis with an inherited abnormality of adenylate cyclase, cyclic AMP phosphodiesterase or the adenylate cyclase receptor. Current evidence, however, suggests that the disturbed cyclic AMP balance may be secondary to some other process—an inhibition of adenylate cyclase function or a stimulation of cyclic AMP phosphodiesterase *in vivo* without a major genetic defect in either enzyme (Voorhees *et al.*, 1973a).

In conclusion, it is perhaps pertinent to comment that the 10-year lag which has been customary before new 'scientific' thought is assimilated into dermatology seems at last to be diminishing. Skin is finally becoming an acceptable tissue for molecular biologists to investigate, and clinicians are becoming more aware that an understanding of many diseases in sufficient depth to lead to better therapy is possible only in molecular terms. If this trend continues, the following decade will be an exciting one for us all. Perhaps the most intriguing line of investigation in this area at the present time is the interrelation between cyclic AMP and cyclic GMP in epidermal physiology (Voorhees *et al.*, 1973a, 1973b; Voorhees *et al.*, 1974a, 1974b). The role of cyclic GMP in epidermal homeostasis will be the subject of a future commentary.

University of Michigan Medical School,
Ann Arbor, Michigan,
U.S.A.

J.J. VOORHEES

Department of Dermatology,
University of Nijmegen,
The Netherlands

P.D. MIER

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Society Proceedings

West of England and Wales Society of Dermatology

5 May 1973, Truro
Chairman: Dr M.HEWITT

Giant Glomangioma DR J.R.SIMPSON

A boy aged $3\frac{1}{2}$ years.

History. He was born 3 weeks premature, weighing 1·9 kg, and was admitted at the age of 6 weeks because of feeding difficulties. These were soon resolved; his general condition and development were found then, as now, to be normal. A birth mark, which had been noticed in the Premature Unit, was seen to have enlarged.

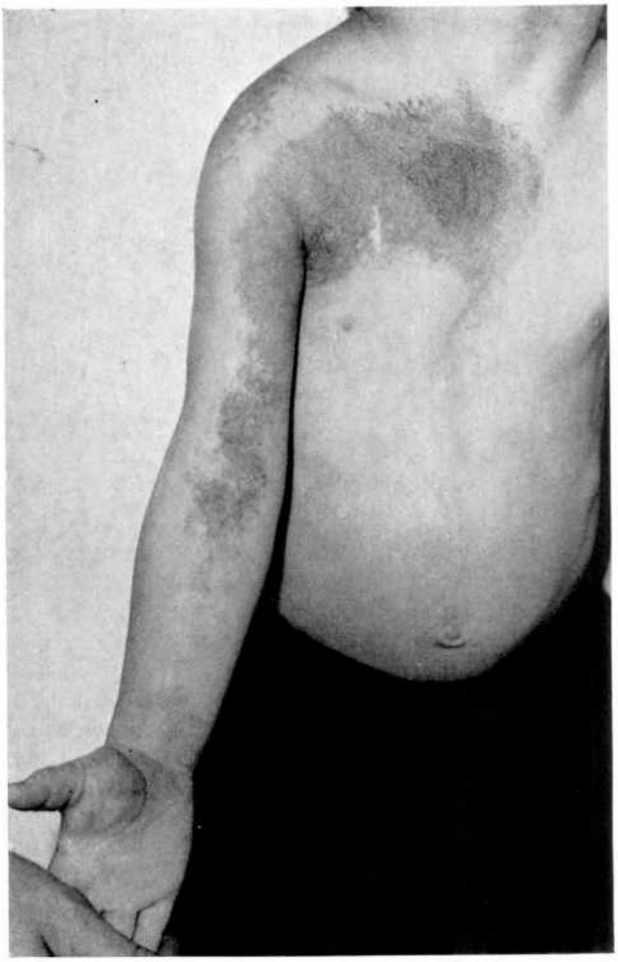

FIGURE 1. Giant glomangioma.

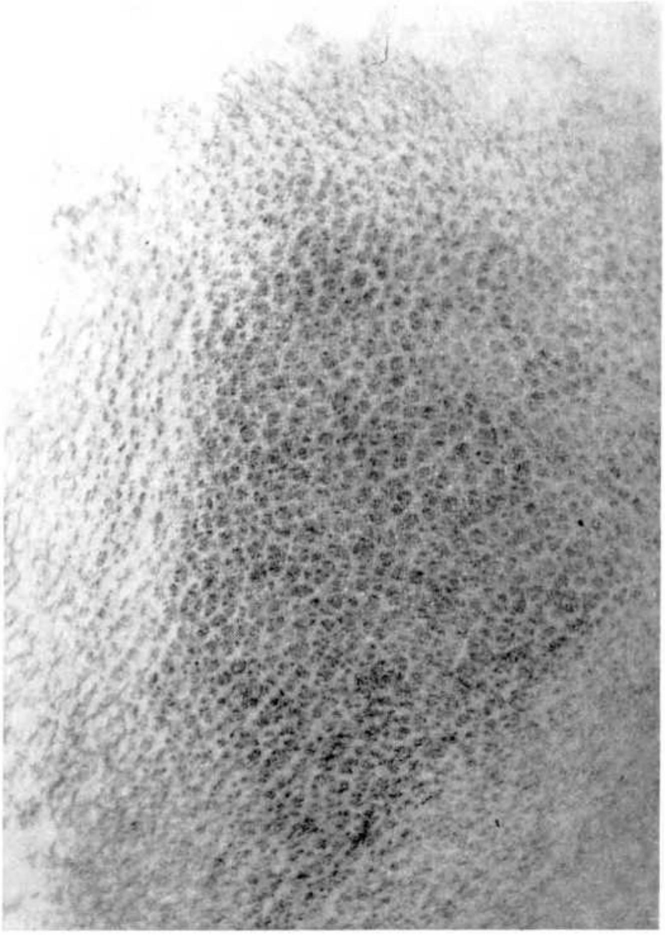

FIGURE 2. Glomangioma showing a reticulated pattern.

To date there has never been any haemorrhage, limitation of function or evidence of pain or tenderness in the affected area, which has extended slightly since infancy.

On examination (1 January 1973). There is discolouration of the skin of the upper sternal and the right pectoral, sterno-clavicular, anterior axillary, anterior brachial and cubital areas, with similar changes on the right acromial region, the front of the right wrist and the right thenar eminence (Fig. 1). The surface is flat and the epidermis is so thin and transparent that the underlying dermis can be seen as a pink background across which lie numerous large, dark-blue vessels. For most of its extent the colour does not fade on pressure but in the centre of the right pectoral area the colour is darker, presents a somewhat reticulated pattern, and fades slightly on pressure; the skin here seems a little thicker. There is no enlargement of the limb or pectoral girdle (Fig. 2).

Histology (10 December 1970). There are multiple dilated blood vessels in the dermis and the upper subcutis, surrounded to a varying degree by typical glomus cells which are also seen in solid masses in the vicinity of such vessels (Fig. 3).

Comment. Although the appearance of such tumours at birth or during infancy is not unknown, and multiple tumours which are often painless are relatively commoner in children, the size of the main lesion in this case appears to be unique.

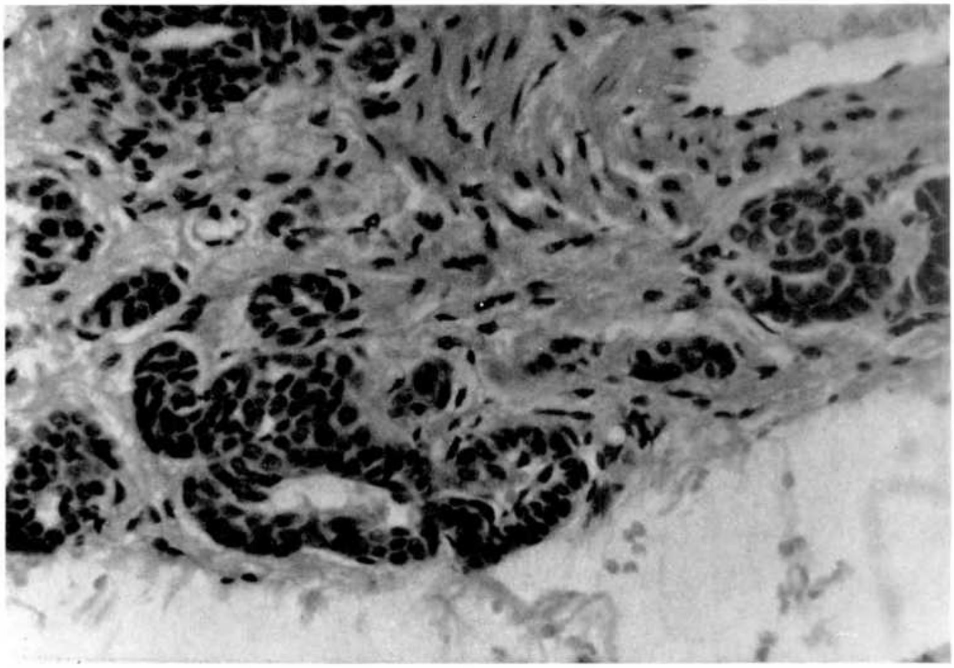

FIGURE 3. Glomangioma. Glomus cells.

Peripilar Keratin Casts DR A.BOWYER

A girl aged 9 years.

History. Streaking along the hair shafts was first noticed in March 1972. The changes grew progressively more extensive, and the school authorities thought they were due to nits. There was no improvement with treatment for head lice. The streaking varies from time to time and is not uniform throughout the scalp hair.

There is no associated skin disease in the child or in the family.

Close examination of the hair shows multiple tubes of keratin around the hair shaft (Fig. 1), the tubes moving freely along the shaft. The only treatment that has helped has been very careful, thorough brushing. These changes are similar to those reported by Kligman (1957) and by Scott (1959).

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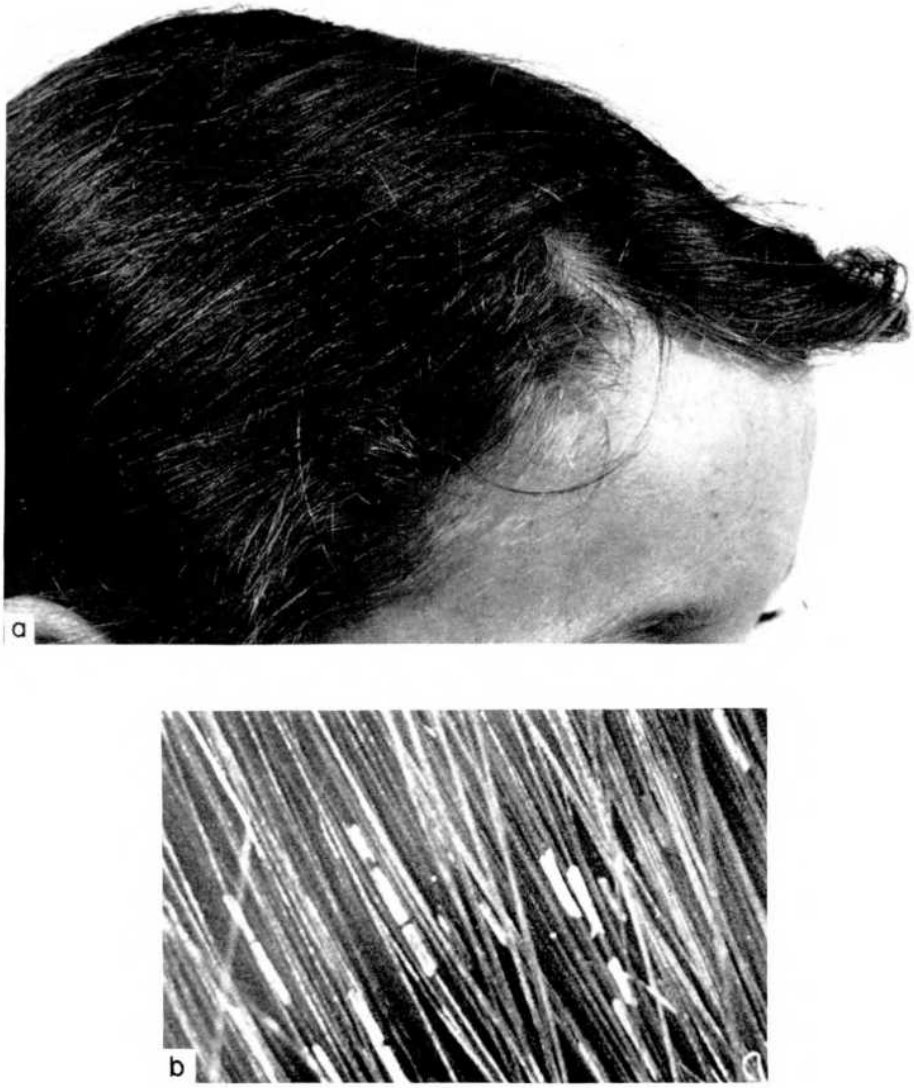

FIGURE 1. (a) and (b). Peripilar keratin casts.

Correspondence

ACQUIRED HYPOMELANOSIS: HYPERPIGMENTATION FOLLOWING REACTIONS TO HYDROQUINONES

SIR, We were interested in the recently reported case of acquired hypomelanosis in a Guyanese (Kelly, 1973). Widespread vitiligo of this type is not uncommon amongst the Indians in South Africa and we too have been unsuccessful in attempting to depigment the areas which are repigmenting. We feel that one statement in the report requires comment as it is quite contrary to our experience. We were surprised to read that 'inflammatory reactions generally precede depigmentation' when hydroquinones are used.

Since 1969 we have carried out a large number of patch tests with preparations containing hydroquinone and monobenzyl ether of hydroquinone as part of our investigation into skin lightening. To date we have applied 3816 tests to 578 individuals, consisting of 200 Asiatics (Indians), 316 Africans (Zulus), and sixty-two Coloureds (mixed white and negro).

We have tested concentrations of the hydroquinones varying from 1% to 7·5% in different bases, creams, ointments and lotions. We use the routine Al-test procedure with adequate controls, the duration of contact when readings are taken being 48 h. The test sites are examined at intervals up to a month to detect delayed reactions. We have found a considerable number of positive reactions, ranging from mild erythema to vesiculation, and, as anticipated, the higher the concentration of hydroquinone, the more severe and frequent the reactions.

In our cases inflammation is invariably followed by *hyper*-pigmentation, and in no instance have we seen depigmentation. This experimental finding is borne out clinically by the large number of patients we see in our clinic suffering from 'skin darkening' following the use of 'skin lighteners'. Vast quantities of these preparations are in use in South Africa for 'improvement' of appearance and elevation of social status, and the additional 'smoothening', as the Africans term it, is highly prized by both male and female purchasers. Although in our opinion more lightening exists in the mind than in the skin, our patients feel that if a preparation 'stings or burns' on application it must be better than one that does not. Consequently, higher and higher strengths are used, applied frequently and vigorously until a good contact dermatitis has appeared. Hypermelanosis follows the erythema and patients then attend the clinic demanding yet another and more powerful 'lightener'.

We are, therefore, unable to accept the statement that 'inflammatory reactions generally precede depigmentation' and would be very interested to have the opinions of other colleagues working among the darker skinned races.

B. BENTLEY-PHILLIPS
MARGARET A. H. BAYLES

Department of Dermatology,
University of Natal, and
King Edward VIII Hospital,
Durban,
South Africa

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PLACEBO RESPONSE TO WHITE SOFT PARAFFIN/PROPYLENE GLYCOL

SIR, Woodford & Barry (1973) found that eight grades of white soft paraffin BP produced definite but transient blanching of normal skin in a test designed originally to assess topical corticosteroid activity. Pallor was more pronounced when the white soft paraffin contained propylene glycol, and it was suggested that this may have been due to the presence of an unknown 'blanching substance' in the white soft paraffin, which was transported into the skin by propylene glycol.

However, it has been suggested that propylene glycol may have an effect on the permeability of the skin barrier (Baker, 1969; Ostrenga *et al.*, 1971). It is conceivable, therefore, that incorporation of propylene glycol in white soft paraffin may lead to modification in the barrier properties of the stratum corneum, facilitating passage of a 'blanching substance' from the white soft paraffin into the skin. The following experiments were performed to assess the significance of such an effect.

Five 10 mg quantities of white soft paraffin, and five 10 mg quantities of white soft paraffin containing 7% w/w propylene glycol were applied by the non-occluded test method to both forearms of ten volunteers, assessment of pallor being carried out as previously described (Woodford & Barry, 1973). The results showed significant differences between the presence and absence of propylene glycol (Table 1, experiment a).

TABLE 1. The effect of propylene glycol on the skin blanching response to white soft paraffin—mean % total possible score for ten volunteers

	Time after application		
	6 h	7 h	8 h
Experiment a			
White soft paraffin	40·3	17·3	2·0
White soft paraffin containing 7% w/w propylene glycol	56·7	26·7	5·3
Significance level*	$P < 0·01$	$P < 0·01$	$P < 0·05$
Experiment b			
White soft paraffin	43·0	19·0	2 0
1 μl propylene glycol followed by white soft paraffin	43·7	20·3	2·3
Significance level*	NS†	NS	NS

* Paired observation analysis by the *t*-test and by the signed-ranks test (Goldstein, 1964). The values for white soft paraffin in the two experiments are not significantly different.

† NS, not significant.

In a second experiment, after allowing the sites to recover for 14 days, 1 μl of propylene glycol was applied, followed 1 h later by 10 mg of white soft paraffin to the same sites. Additional sites were tested with white soft paraffin alone, as before. There was no significant difference between the results obtained in the presence and absence of propylene glycol (Table 1, experiment b), indicating that application of propylene glycol did not affect the subsequent blanching response to white soft paraffin.

These results tend to substantiate the suggestion that propylene glycol increases white soft paraffin-induced pallor by acting as a carrier for a 'blanching substance', rather than by decreasing the barrier function of the stratum corneum.

R. WOODFORD
B.W. BARRY

School of Pharmacy,
Portsmouth Polytechnic,
Portsmouth, Hants

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HERPES ZOSTER OF THE ANOGENITAL AREA AFFECTING URINATION AND DEFAECATION

SIR, I should like to comment on the statement of Fugelso *et al.* (1973) that only 'twenty-two cases of herpes zoster with bladder symptoms have been reported, all in urological journals'.

Although herpes zoster of the external genitalia and urethra is not uncommon, involvement of the bladder is rare, indeed Head (1910) did not observe any cases of intra-urethral or intra-vesicular herpes zoster in his classical studies on 416 cases of the disease.

However, reports other than those mentioned by Fugelso *et al.* (1973) are found in the literature.

Colcott Fox (1898) claimed to have seen a case in which involvement of both buttocks together with varioliform lesions was seen in an infant.

Darget (1929) reported a case in a man, aged 65, who presented with haematuria and herpes zoster on the course of the ilio-inguinal nerve. Cystoscopy confirmed vesicles on the bladder wall. He also noticed another case but without skin lesions.

Chesterman (1932) published a case in a woman, aged 64, who had severe pain on micturition and zoster lesions below the left buttock.

Rinker (1941) reported a case similar to that of Darget's second case of herpes zoster of the bladder without accompanying skin lesions.

Harkness (1950) mentioned two cases of his where endo-urethral involvement was found causing an abacterial urethritis with characteristic skin lesions on the inner aspect of the thigh.

M.A.WAUGH

Department of Venereology,
Charing Cross Group of Hospitals

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Book Reviews

The Physiology and Pathophysiology of the Skin. VOLUME 1. **The Epidermis.** VOLUME 2. **The Nerves and Blood Vessels.** (Ed.) A.JARRETT (1973). London and New York: Academic Press. Pp. xxxiv+xxxix+806, illustrated. Price £7.50 and £9.80.

These two volumes together clock up 806 pages, and it appears from the text that further works, to include pigmentation and hair, are planned. The series must, therefore, be viewed as a potential successor to Rothman's 'Physiology and Biochemistry of the Skin' and, in bulk at least, as a rival to 'Advances in Biology of the Skin', albeit the Montagna series comprises symposium volumes rather than constitutes a planned textbook.

'The Epidermis' is written entirely by the editor, Dr A.Jarrett. The first chapter deals with histology, ultrastructure, dermo-epidermal interactions, dendritic cells and wound healing; subsequent chapters are devoted to 'Biochemistry', 'Epidermal Kinetics', 'Comparative and Experimental Keratinization' and 'Chemistry of Keratin', and finally there are four chapters on the abnormal human epidermis.

There are at least two ways of writing a textbook, neither without merit. At one extreme, the conservative approach includes only material which is well established and can be conveniently structured; at the other, the catholic approach explores every avenue. Jarrett clearly veers towards the latter. In the section on epidermal kinetics, for example, he properly describes cell division, the cell cycle, measurement of cell turnover times, the transit of keratinocytes within the epidermis, and the kinetics of hyperplasia. He discusses the interesting phenomenon of diurnal rhythms in the epidermis, as well as the important problem of ageing and the role of genetic and environmental factors. On a more controversial level, having devoted several pages to chalones and—by way of balance—one page to the epidermal growth factor, he cannot resist committing to print the ingenious speculation that adenyl cyclase may be chalone and that its loss could account for the increase in cellular glycogen which occurs after injury.

In the fields of his own interest, the author expectedly reveals his personal, and at times controversial, convictions. Tonofibrils receive short shrift as precursors of or contributors to epidermal keratin: 'similar fibrils exist in a wide variety of cells which never produce keratin'. And clearly Jarrett believes that some, at least, of the DOPA negative dendritic cells in the upper epidermis, are not Langerhans' cells but 'previously functioning melanocytes'. He argues that such high level dendritic cells are associated with the type of epidermal keratin produced, and appears to imply that they could in some way act as mercurial messengers arriving from the dermis.

In Volume 2, Professor D.C.Sinclair and Dr T.J.Ryan deal respectively with nerves and blood vessels. Sinclair's section appears, at least to this nervously ignorant reviewer, to be monumental, and I suspect may prove unique in its field. The anatomy of the cutaneous fibres and the end-organs is clearly explained with good diagrams and excellent micrographs. Sections follow on the electrophysiology and psychophysiology of cutaneous sensation, on motor nerves and reflexes, and on pathophysiology. As an example of the succinctness of Sinclair's review of the vexed problem of cutaneous sensation, I quote one phrase: 'It now appears certain that stimulus-specific endings exist in the skin, but whether arousal of these endings can give rise to a particular sensation is still unknown'.

Ryan's contribution is similarly comprehensive, dealing not only with the structure, pattern and development of the cutaneous vessels, but also with blood flow, permeability, vascular responses, inflammation and factors influencing growth of vascular endothelium.

The volumes are well produced, and very conveniently referenced throughout by the use of footnotes on each page. If the approach to the material is not, perhaps, equally critical in all the sections, the reader can thus quickly track down original sources.

F.J.G.EBLING

Dermatology. P.HALL-SMITH, R.J.CAIRNS AND R.L.B.BEARE (1973) 2nd edition. London: Crosby Lockwood Staples. Pp. 374. Price £7.00.

This book claims to be a concise account of modern thought in dermatology, and the tables, diagrams and photographs are designed to help the M.B. and M.R.C.P. candidate. It largely succeeds in these aims. The format is clear and helpful, both for continuous reading and for quick assimilation of information on any one topic. In this respect it is the most successful of the texts at present available. However, it does not meet the demand for a cheap text for students and must as a consequence fail in its chief aim of capturing the undergraduate market.

The balance between itemization of specific diseases and review of certain aspects of the management of the skin is excellent. 'Therapeutic Guide, 'Surgical Treatment' and 'The Skin in Systemic Disease' are useful brief essays.

Conciseness sometimes gives rise to half truths, which are misleading and which matter most in the management of the patient. Few patients receiving methotrexate will agree to a liver biopsy every 6 months. Stopping dapsone when a patient with leprosy has a flare-up may not be in his best interests. Hexachlorophane powder is not to be recommended, *particularly* for neonates. Some practitioners do not approve of 1% adrenaline in local anaesthetics. Few will use thyroid 30 mg twice daily in the treatment of atopic eczema.

T.J.RYAN

Year Book of Dermatology 1973. F.D.MALKINSON AND R.W.PEARSON (Eds.) (1973) Chicago: Year Book Medical Publishers (U.K. distributors, Lloyd-Luke, London). Pp. 396, 60 figures. Price £7.75.

This latest edition of the Year Book covers the world literature for the year 1972. It is introduced by a comprehensive review of 'Immunofluorescence in Dermatology' by W.Mitchell Sams. The clearly written abstracts are conveniently classified as in previous years. At the end of each section of abstracts there is a useful list of additional articles recommended to the reader.

There is little to criticize in this competently edited volume, which has been published with commendable speed. The editorial comments are well-informed and interesting. Once again the Year Book must be highly recommended.

ARTHUR ROOK

British Journal of Dermatology (1974) **90**, 239.

*Reviews**

DRUG REACTIONS XXIV

Cutaneous reactions to cytostatic agents

The use of cytostatic agents has increased considerably over the last decade. These agents not only are used for the treatment of malignant disease, particularly leukaemia and the reticuloses, but also have become established in the treatment of non-malignant conditions. These include auto-immune disorders such as systemic lupus erythematosus, rheumatoid arthritis and pemphigus, immunosuppression associated with transplantation surgery, and miscellaneous conditions including the nephrotic syndrome and psoriasis.

Toxicity from these drugs occurs frequently, the major effects being noted on organs whose cells are dividing rapidly. The most important of these effects is bone marrow depression, the ensuing thrombocytopenia manifesting as a purpura, or haemorrhagic necrosis which, on pressure points, may lead to ulceration.

These drugs reduce the body's natural resistance to infection whether it is bacterial, viral or fungal (including yeasts), resulting in overt infection which can be widespread and severe. Localized herpes zoster may in such situations become generalized, haemorrhagic and lethal, and be indistinguishable from severe varicella. In the same fashion, vaccination can be followed by vaccinia, and a localized herpes simplex infection may become generalized. Recently a patient developed Norwegian scabies while receiving azathioprine after renal transplantation (Paterson, Allen & Beveridge, 1973).

Cytostatic agents may be classified into alkylating agents, e.g. busulphan, chlorambucil, cyclophosphamide, melphalan and nitrogen mustard; alkaloids, e.g. vincristine; antimetabolites, e.g. 6-azauridine, cytosine arabinoside, 5-fluorouracil, 6-mercaptopurine and methotrexate; antibiotics, e.g. bleomycin, daunorubicin and actinomycin D; miscellaneous, e.g. L-asparaginase, hydroxyurea, procarbazine and colchicine.

Alopecia

All of these drugs may produce some degree of alopecia. This has been discussed in detail in a previous review (Levantine & Almeyda, 1973). The drugs induce an anagen alopecia, interfering with hair growth at the proliferative phase. Regrowth of hair occurs if the drug is stopped and occasionally if the dose is reduced.

Pigmentation

Certain of the cytostatic agents produce pigmentation.

Busulphan induces a diffuse, brown pigmentation of the face, forearms, chest and abdomen (Galton, 1953). This adverse reaction has been reported to occur in 5–10% of patients, particularly in those with a dark complexion (Moller, 1966). Less commonly, Addison's disease may be simulated (Kyle *et al.*, 1961; Harrold, 1966; Feingold & Koss, 1969). These patients are hyperpigmented and complain of severe weakness, fatigue, anorexia, nausea and weight loss. Buccal pigmentation is uncommon and palmar creases are normal. Investigations do not show hypo-adrenalism. The mechanism of this reaction is uncertain. Kyle *et al.* (1961) postulated that busulphan inactivates sulphydryl groups in the skin by 'dethiolation', which removes an inhibitor of tyrosinase. Histologically, there is increased melanin in the basal layer of the epidermis and in the dermal melanophores.

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* In future the regular review articles on Contact Dermatitis, Therapeutics and Drug Reactions will be published only twice yearly to allow the introduction of regular reviews on three other topics.

Cyclophosphamide has produced hyperpigmentation of the skin, accentuated in exposed areas, in children with nephrotic syndrome (Salazar, Bercowsky & Lara, 1969). A 3-year-old boy with the nephrotic syndrome developed brown discolouration at the base of the finger- and toe-nails after treatment with this drug. He also had a brown line on the teeth at the gingival margin (Harrison & Wood, 1972). Melanosis of the nails was described also as a rare concomitant of cyclophosphamide therapy in Bantu patients (Falkson & Schultz, 1960).

Bleomycin, which is used in the treatment of various reticuloses, may cause hyperpigmentation of the skin lesions (De Bast *et al.*, 1971). Excoriations may also become pigmented (*British Medical Journal*, 1972).

Systemic 5-*fluorouracil* may produce diffuse melanosis of the nail plate (Falkson & Schultz, 1962) and occasionally there may be alternate pigmented and non-pigmented bands corresponding to each course of treatment.

Stomatitis

Stomatitis may result from any of the cytostatics. This may or may not be associated with agranulocytosis. Staley *et al.* (1961) reported this side effect occurring to a varying degree in two-thirds of cancer patients treated with systemic 5-*fluorouracil*. In several patients a pseudomembrane formed over much of the mucosal surface of the lower lip. *Bleomycin* commonly causes mucosal lesions although it lacks toxicity to the haemopoietic system (*British Medical Journal*, 1970).

Reactivation of radiation dermatitis

Many cytostatic drugs are capable of inducing an inflammatory reaction in previously irradiated skin. This has so far been reported with *vinblastine*, *actinomycin-D*, 5-*fluorouracil*, *hydroxyurea and methotrexate*. The phenomenon, which has been reproduced with methotrexate in both man and guinea-pigs, results from reactivation of an already receding radiation dermatitis (Möller, 1970).

Photosensitivity

Photosensitivity following the use of systemic 5-*fluorouracil* was reported in detail by Falkson & Schultz (1962). Ten of sixty-four patients showed erythema after a suberythema exposure to sunlight. The erythema appeared within half an hour of exposure and persisted for several days, even without subsequent exposures. In one patient a polymorphic light eruption consisting of a plaque-like rash, most marked in a butterfly distribution, developed with each course of 5-fluorouracil. Marked pigmentation, far in excess of that expected, was noted in several patients. The pigmentation appeared within half an hour of exposure to sunlight. Roenigk, Fowler-Bergfeld & Curtis (1969) reported a 5% incidence of photosensitivity in patients receiving weekly oral doses of methotrexate for psoriasis.

Erythema multiforme-like eruptions

Brauer, McEvoy & Mitus (1967) described erythema multiforme developing on two occasions following administration of nitrogen mustard to a patient with Hodgkin's disease. A 35-year-old man developed an erythema multiforme-like eruption while receiving busulphan for chronic granulocytic leukaemia (Dosik *et al.*, 1970). Erythema multiforme has also been reported with cyclophosphamide therapy (Falkson & Schultz, 1962).

Carcinogenesis

It is generally accepted that cytostatic agents, particularly the alkylating agents, are potential carcinogens (Byland, 1964). Feingold & Koss (1969) reported a patient who developed carcinoma of the vulva after 6 years of busulphan therapy for chronic myeloid leukaemia. The possibility of the carcinoma being secondary to busulphan treatment was discussed.

Gynaecomastia

Gynaecomastia has been ascribed both to *busulphan* (Galton, Till & Wiltshaw, 1958) and to *vincristine* therapy (Smith & Barrett, 1967).

Miscellaneous

There are numerous reports of morbilliform eruptions and unspecified 'dermatitis' occurring with various cytostatic agents. Hypersensitivity reactions are said to be not infrequent with *azathioprine* (Adams, Gordon & Maxwell, 1967) and L-*asparaginase* (Oettgen & Schulten, 1969). Knisley, Settipane & Albala (1971) described a severe hypersensitivity reaction in a patient receiving *chlorambucil.*

Other isolated cutaneous reactions have been reported and often associated with individual agents. Kyle & Dameshek (1964) associated porphyria cutanea tarda with *busulphan* therapy in a man receiving the drug for chronic granulocytic leukaemia.

Unusual reactions have been reported in patients receiving *aminopterin* for psoriasis. In one patient, after 1 week's treatment, the psoriatic lesions turned into painful erosions which bled at the slightest touch (Oswald, 1968). Gubner (1951) described haemorrhagic crusting of psoriatic lesions concurrent with cessation of scaling. Another report described erosions about the genitalia occurring whilst the psoriasis improved (Rees *et al.*, 1964). *Methotrexate* has also been reported to cause ulceration of psoriatic lesions (Roenigk *et al.*, 1969).

Bleomycin may produce rashes in 10% of patients (*British Medical Journal*, 1970). Late skin lesions may be more specific, occurring on the hands and elbows and less often on the back. They take the form of a polymorphic erythema or hyperkeratosis, sometimes progressing to necrosis.

Actinomycin D produced an acneiform eruption in nine of eleven adult patients. It spread from the face to the trunk (Epstein & Lutzner, 1969).

The Middlesex Hospital, ASHLEY LEVANTINE
London, W.1
The Enfield Group of Hospitals JOHN ALMEYDA

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Michelson Memorial Meeting

The first Dr Henry E. Michelson memorial meeting will be held in conjunction with the Minnesota Dermatological Society meeting at the University of Minnesota, Minneapolis, on 17 and 18 May 1974. Guest speakers will be Dr Walter Shelley of the University of Pennsylvania and Dr Richard Winkelmann of the Mayo Clinic Medical School. For further information, contact Dr Harry Irving Katz, Secretary, Minnesota Dermatological Society, 2160 Cavell Avenue North, Minneapolis, Minnesota 55427, U.S.A.

International Health Foundation Award 1973

We are pleased to announce that the 1973 Award of the International Health Foundation (venereal disease section) will go to Dr William F. Felton of St Thomas' Hospital, London, for his paper 'The location of patients and venereal disease control'. The Award is worth 5000 Swiss francs.

The subsidiary Award of 2000 Swiss francs for the best paper received from an author under 35 years of age will go to James M. Stoffels Jr and Ben A. Say of the U.S. Public Health Service Center for Disease Control, Atlanta, currently working at the Baltimore Health Department in Maryland, who wrote on 'Gonorrhea group advisory sessions—no field follow-up'.

In addition, the judges have asked that special mention be made of a paper entitled 'The control of venereal disease' by Dr John W. R. Harris of the Royal Infirmary, Sheffield.

Skin Microbiology Club

The thirteenth meeting of the Club took place at St John's Hospital, Leicester Square, on 5 December 1973, when Dr A. C. Baird-Parker reviewed the classification of the genus *Staphylococcus* in the light of a revision of the genus to be published in 1974. The revision removes anomalies from the scheme proposed by Baird-Parker in 1963, which has become the standard classification of the *Staphylococcus* sp. The original scheme identified minor pathogens amongst the coagulase negative species; B-P S II (biotype I) strains appearing in bacteraemia, colonized/infected prostheses and in acne lesions more frequently than did other biotypes.

Discussion covered the difficulties of producing a scheme which had clinical significance and taxonomic value, but was easy and quick to perform in a routine laboratory; the genetic stability of the characters investigated; and the advisability and validity of applying identification schemes devised for food hygiene or veterinary work to medical, especially dermal microbiology.

The Club, which is now in its fifth year, meets twice in central London and once elsewhere each year; joint meetings have been held with the Dowling Club and with the East Midlands Microbiology Society. Details of future meetings may be obtained from the Convenor, Dr W. C. Noble, Department of Bacteriology, St John's Hospital for Diseases of the Skin, Homerton Grove, London E9 6BX.

Italian Course on Biological Research in Relation to Dermatology

The First Italian National Course on Recent Biological Research in relation to Dermatology will be held in Pavia on 9–13 September 1974. Details may be obtained from Professor D. Cerimele, University Dermatological Clinic, Policlinico, 27100 Pavia, Italy.

APPOINTMENTS

Woolwich Group of Hospitals

Dr Harold Woolfson, recently appointed Consultant Dermatologist to the Woolwich Group of Hospitals, qualified in medicine in Glasgow in 1950, and after junior hospital posts he entered general practice in 1953. He was also appointed Physician Superintendent to East Park Home for Infirm Children in 1961, and in 1964 he gained the Diploma of Child Health. The M.R.C.P. of both Edinburgh and Glasgow followed in 1967. He became a Registrar in Dermatology in Stobhill General Hospital in 1969 and in 1971 Senior Registrar in Dermatology to the Glasgow Teaching Hospitals. His particular interests have been the skin problems of physically handicapped children, the treatment of skin malignancies, and the immunological aspects of leukoderma. Publications include 'Clinical studies of skin changes in handicapped children'.

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Edited by

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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Robert Harold Champion M.A. M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D.(Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Niels Hjorth M.D.(Copenhagen)
William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
Francisco Kerdel-Vegas M.D.(Caracas) M.SC.(N.Y.)
Gunnar Lumbolt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
Stephen Owen Bellamy Roberts M.A. M.B. B.CHIR.(Cantab.) M.R.C.P.(Lond.)
Arthur James Rook M.D.(Cantab.) F.R.C.P.(Lond.)
Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
Peter Derrick Samman M.A. M.D.(Cantab.) F.R.C.P.(Lond.)
Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
Ian Bruce Sneddon M.B. CH.B.(Sheffield) F.R.C.P.(Lond.)
Darrell Sheldon Wilkinson M.D.(Lond.) F.R.C.P.(Lond.)
Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
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microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometer(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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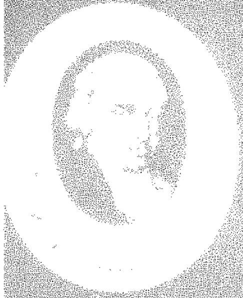

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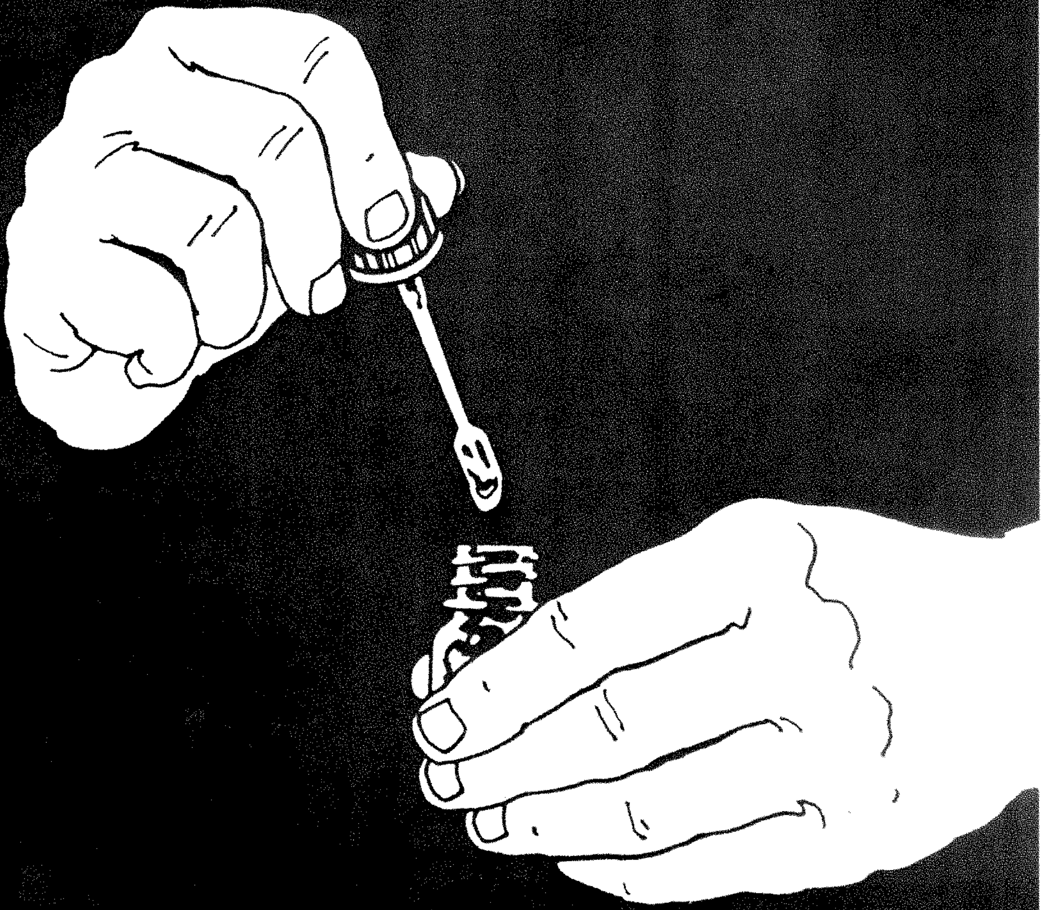

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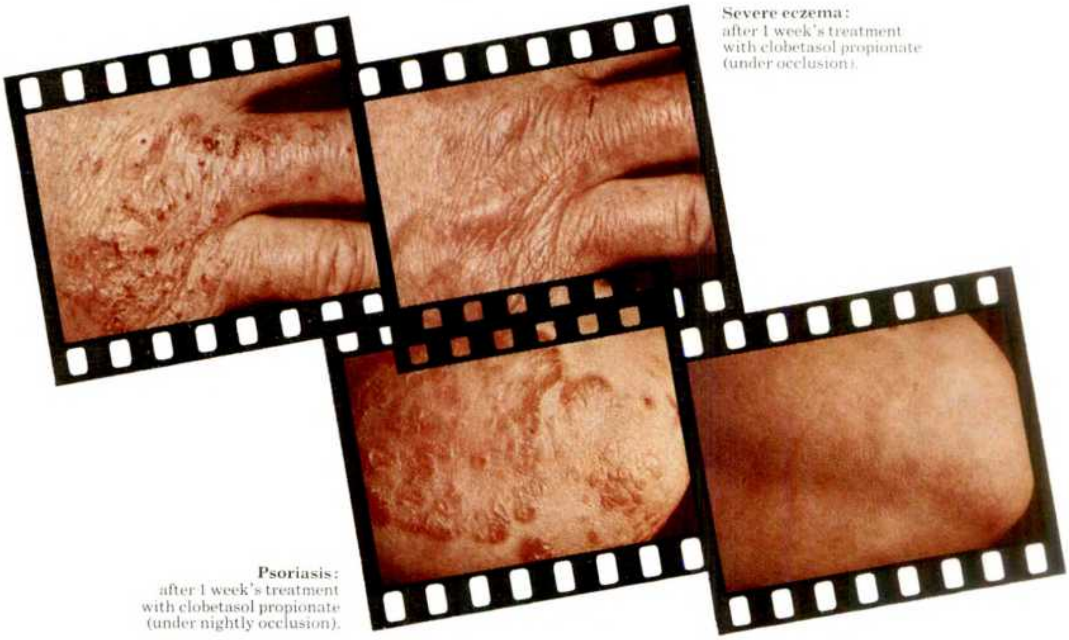

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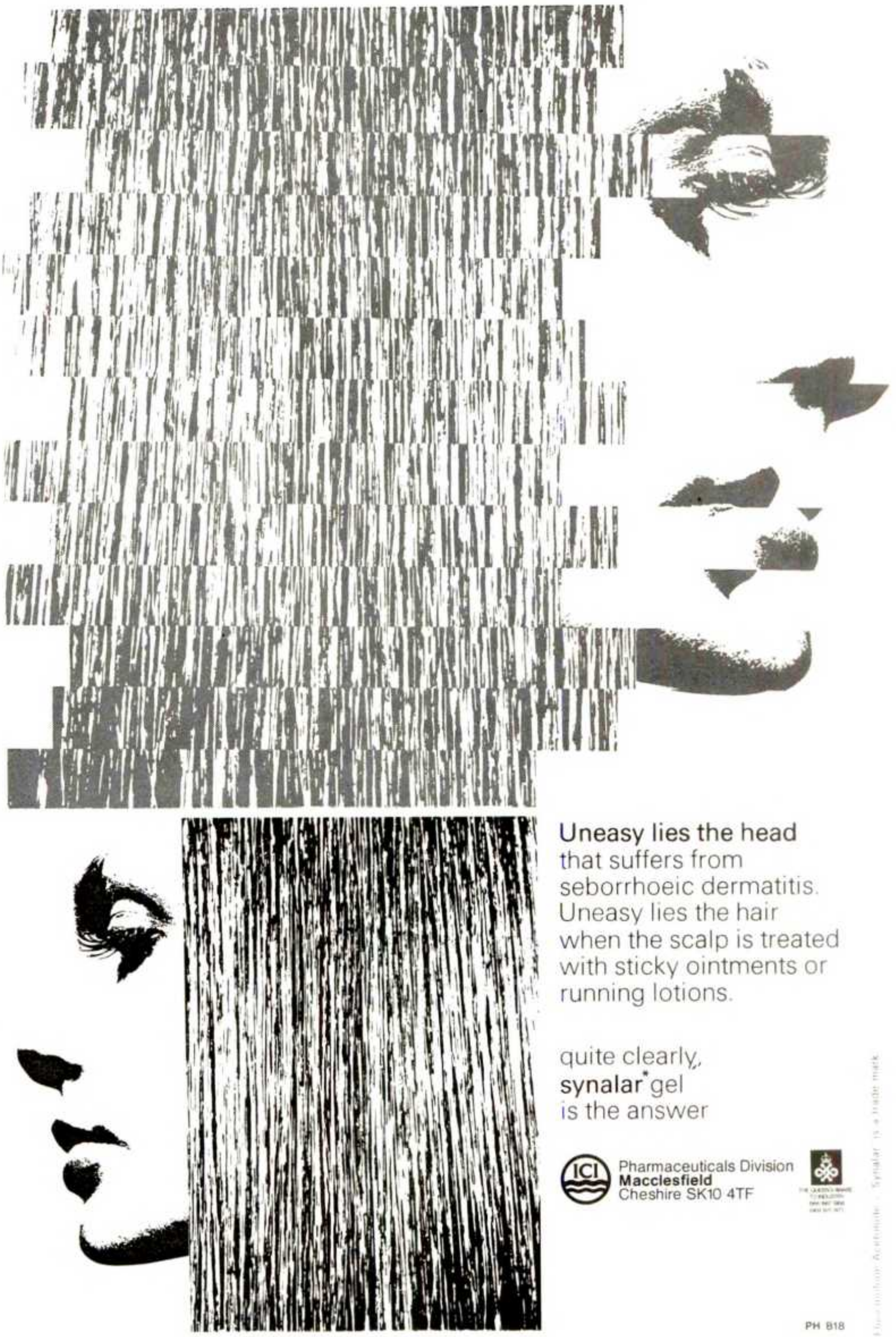

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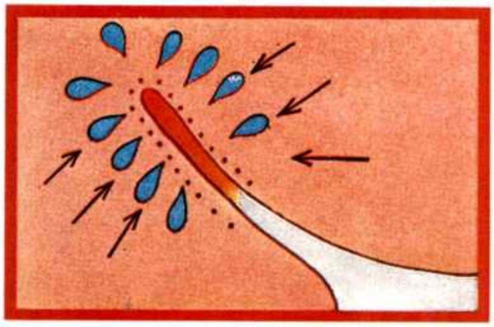

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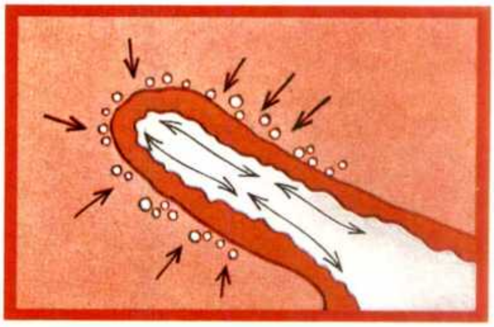

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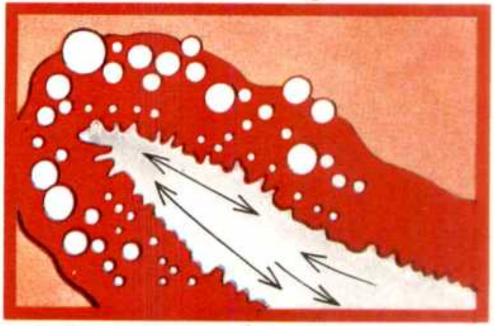

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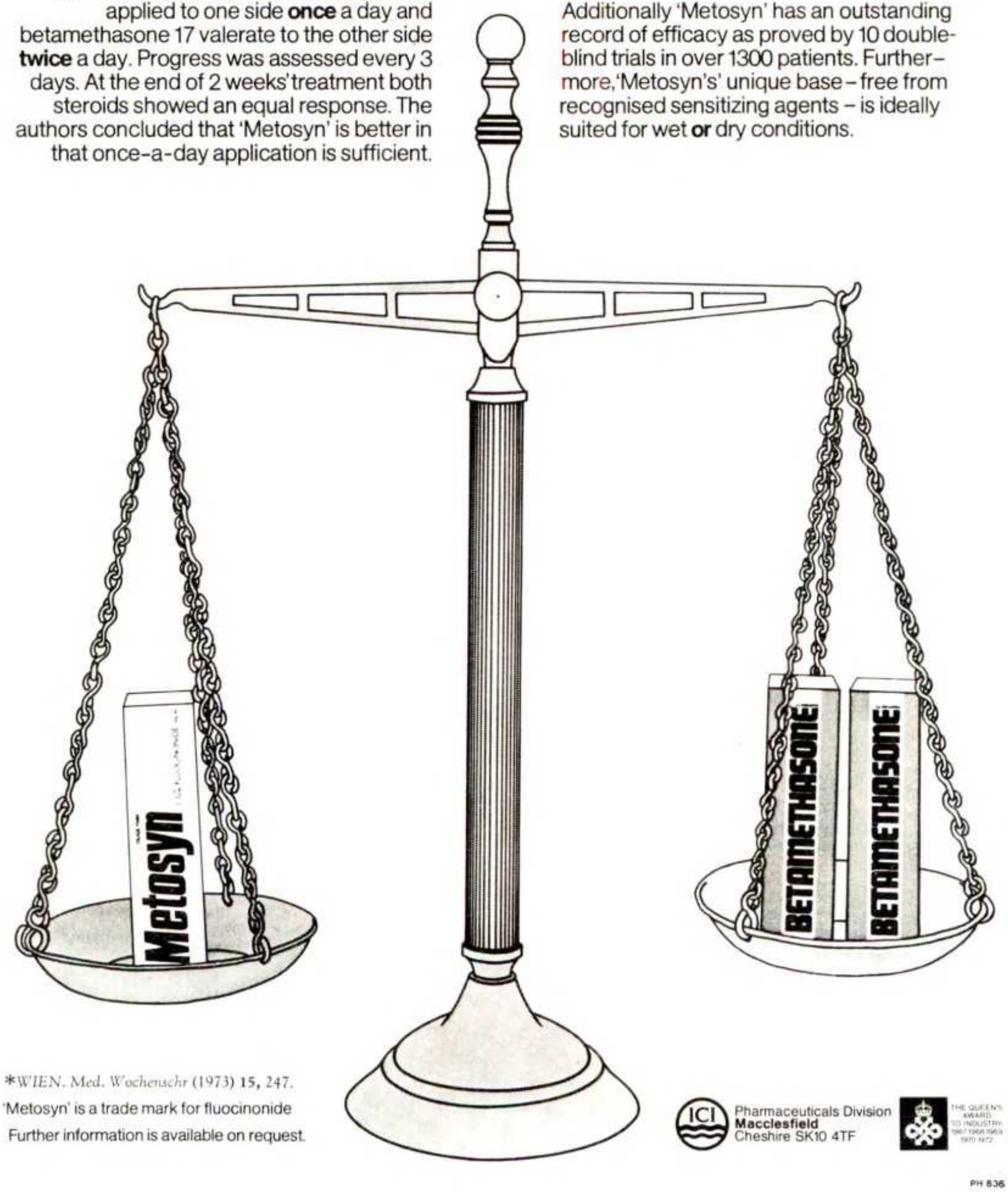

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British Journal of Dermatology (1974) **90**, 365.

Clinical and Laboratory Investigations

An immunofluorescence study of lichen planus

E.H.BAART DE LA FAILLE-KUYPER AND H.BAART DE LA FAILLE

Department of Dermatology (Professor Dr L.H.Jansen), University Hospital, Catharijnesingel 101,
Utrecht, The Netherlands

Submitted 24 April 1973; Accepted for publication 3 November 1973

SUMMARY

In the present immunofluorescence study we report the occurrence of fibrin or related substances and of IgM in the upper part of the dermis in all forty lichen planus lesions examined. Different amounts of various complement components and fragments were located in the epidermal basement zone, and in the colloid bodies. IgG, IgM, IgA and fibrin were also found in the colloid bodies, sometimes together with albumin.

The earliest pathological change, which was seen in perilesional skin and in clinically normal skin from sites of predilection, was the presence of colloid bodies and pigment containing macrophages in the upper dermis. Deposition of fibrin appeared to be another early sign, as it was discernible in areas devoid of inflammatory infiltrate. The possible significance of these findings is discussed.

In previous immunofluorescence (IF) studies, Baart de la Faille-Kuyper (1969, 1972) has been able to demonstrate fibrin or related substances (further designated as fibrin) within the superficial dermis in all the lichen planus (LP) lesions investigated. These findings have been confirmed by other workers (Barthelmes & Haustein, 1970; Copeman, Schroeter & Kierland, 1970; Ebner & Kraft, 1972). Baart de la Faille-Kuyper (1969, 1972) has also located immunoglobulins in the epidermal basement zone (EBZ), a finding not in accordance with the results reported by Barthelmes & Haustein (1970). Copeman *et al.* (1970), however, have mentioned the presence of IgM, and β_1C/β_1A in the EBZ in some of their cases of LP.

In three patients with bullous LP, a linear deposition of IgG and C4/C3 has been found in the EBZ in peribullous areas (Baart de la Faille-Kuyper, 1972). An identical staining pattern has been observed in the clinically uninvolved skin of two of these patients. Complement fixing IgG antibodies with affinity for human EBZ antigens were demonstrable in the serum of one patient.

Various plasma factors have been detected in the colloid bodies (Ueki, 1969; Baart de la Faille-Kuyper, 1969, 1972; Barthelmes & Haustein, 1970; Ebner & Kraft, 1972).

This report extends the earlier IF findings.

MATERIALS AND METHODS

The material consisted of biopsies from lesions and from clinically uninvolved skin (extensor surface of forearm) of twenty-two female and eighteen male patients with LP. In five cases, biopsies were also taken from the uninvolved skin of the wrists. The biopsies were obtained from various types of skin lesions, care being taken to include perilesional skin. The duration of the cutaneous manifestations varied from 1 day to 10 years.

The following antisera and conjugates were used for IF staining: rabbit antisera to human IgG, IgA, IgM, albumin, C1q, C4, C3b, C3c, C3d, C5 and C4+C3c+C3d (C4/C3), and a FITC-labelled horse anti-rabbit globulin serum, were obtained from the Netherland's Red Cross Blood Transfusion Service, Amsterdam. The anti-fibrin(ogen) conjugate was obtained from Nordic Pharmaceuticals and Diagnostics, Tilburg, The Netherlands.

The methods of preparation and the specificity of the antisera to the complement components and fragments have been published by Engelfriet *et al.* (1970), and by van Joost, Cormane & Pondman (1972). The characteristics of the other reagents of the Blood Transfusion Service have been described elsewhere (Luyten & Baart de la Faille-Kuyper, 1972). The characteristics of the anti-fibrin(ogen) conjugate are: protein concentration 10 mg/ml; mol. F/P ratio 5:5; precipitation titre in agar block titration 1/64; dilution 1/30.

The sera of the patients were tested on the following substrates: skin from healthy individuals of blood group O, guinea-pig lip, and rat liver.

Besides a Zeiss Standard GFL microscope equipped for darkfield immunofluorescence (light source HBO, 200W; exciter filter BG 12/3 mm; barrier filters 44, 45, 47), we also used a Leitz Orthoplan microscope with epi-illumination and interchangeable dichroic mirrors, equipped with a Xenon arc (XBO, 75W) (Ploem, 1967). We used position 3 of the vertical illuminator, and K 515 as an extra barrier filter (Ploem, 1967).

RESULTS

The localization of plasma factors in the cutaneous tissues

The epidermal basement zone. Fibrin could be demonstrated in the EBZ in all forty lesions examined, the deposition being variable in quantity. In fully developed lesions, fibrin occurred as a broad 'band' from which numerous thread-like outgrowths extended into the dermis, and to a minor degree also into the epidermis (Fig. 1). Especially at low magnification, such a band accentuated the characteristic 'saw-toothed' rete pegs. In epidermal lacunae we observed dense networks of fibrin, in which colloid bodies could be found entangled.

Fibrin deposition was far less marked in newly developing lesions and in areas with minor signs of inflammation. At the edges of lesions devoid of any cellular infiltration, fibrin occurred in an interrupted pattern. Fibrin was absent in clinically uninvolved skin.

IgM was found in all lesions, usually in a finely granular pattern (Fig. 2). The quantity of IgM was variable; IgM deposits were mostly restricted to more or less small areas, where granules could often also be found among epidermal and inflammatory cells.

IgA was never detected, whereas linear deposition of IgG was encountered in small areas of the EBZ near epidermal lacunae.

Staining for C4/C3 resulted in a conspicuous immunofluorescence in all lesions. In biopsies from twelve patients the presence of other complement components and fragments was sought. C1q was never detectable, trace amounts of C4 were found in all cases, and rather coarse granules of C5 were observed in two biopsies. C3d was always found in abundance (Fig. 3), usually partly in a linear, partly in a granular pattern. Much smaller quantities of C3c could be detected, whereas staining for C3b was negative.

The results obtained in uninvolved skin were similar to the findings in the skin of healthy individuals (Baart de la Faille-Kuyper, van der Meer & Baart de la Faille, 1974).

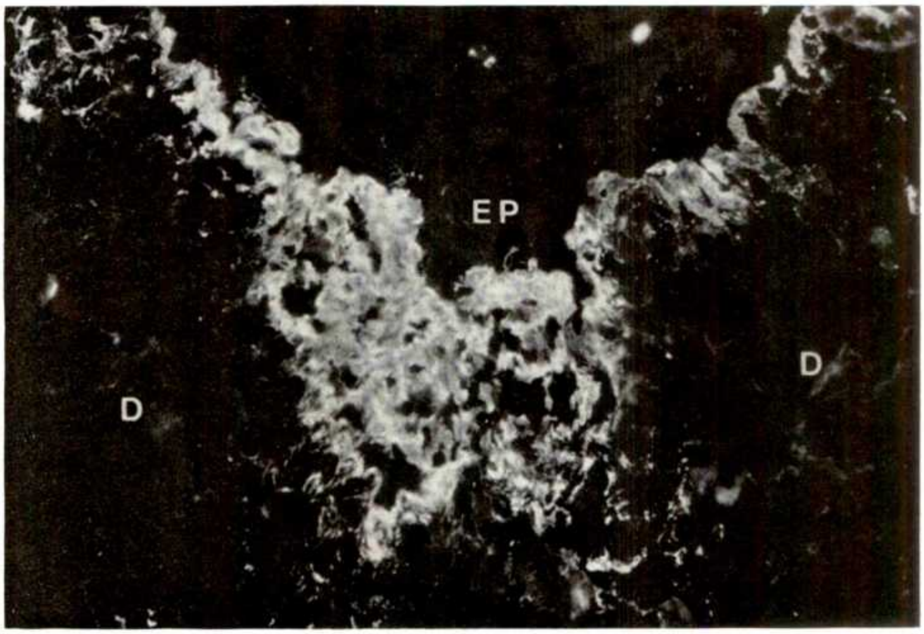

FIGURE 1. LP skin lesion showing extensive deposition of fibrin in the upper part of the dermis. EP, epidermis; D, dermis (Zeiss Standard GFL microscope, × 16 neofluar).

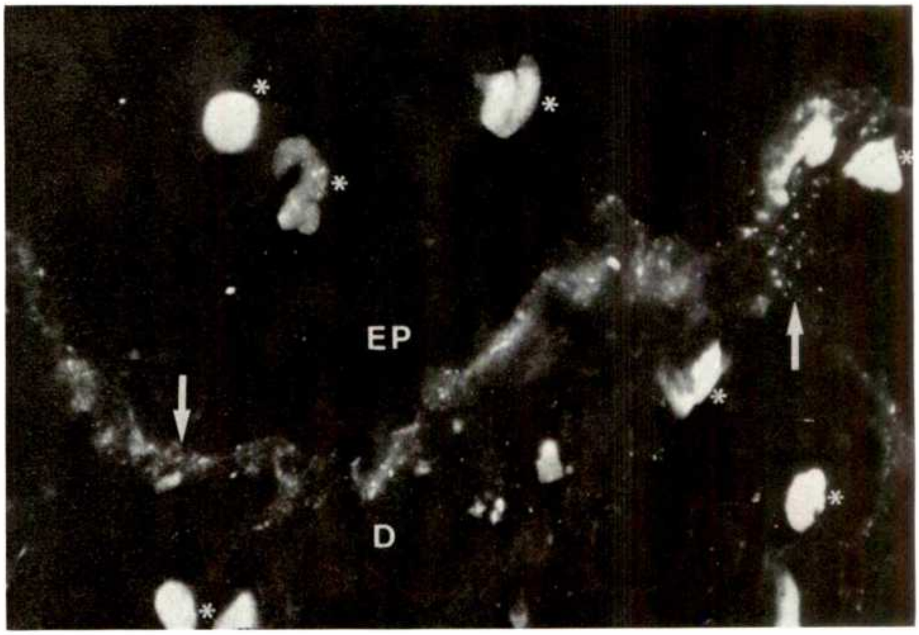

FIGURE 2. Granular deposits of IgM in the epidermal basement zone (arrows), and in a number of colloid bodies (asterisks). EP, epidermis; D, dermis (Leitz Orthoplan microscope, × 54 oil immersion).

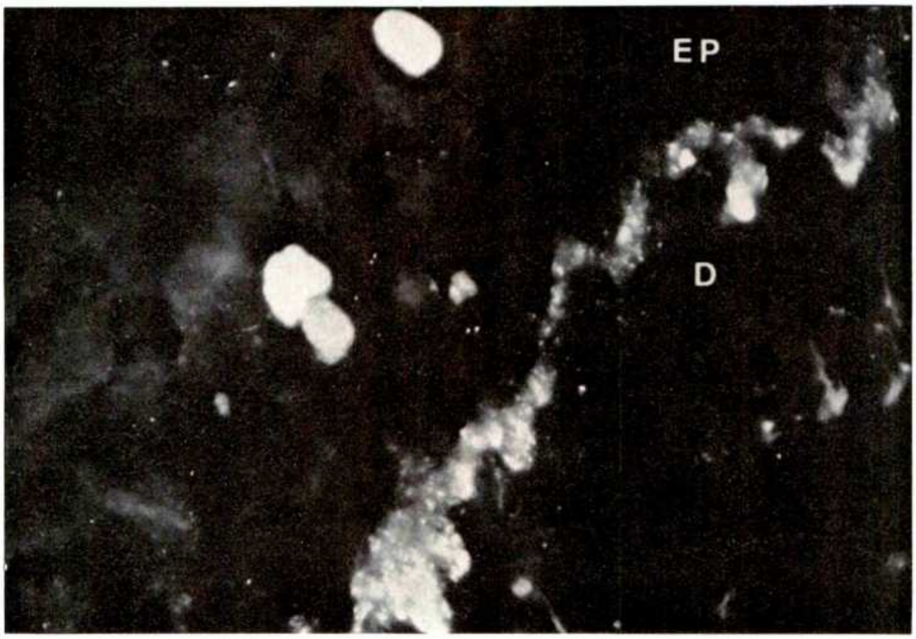

FIGURE 3. LP lesion showing the presence of C3d in the epidermal basement zone and in colloid bodies. EP, epidermis; D, dermis (Zeiss Standard GFL microscope, × 40 neofluar).

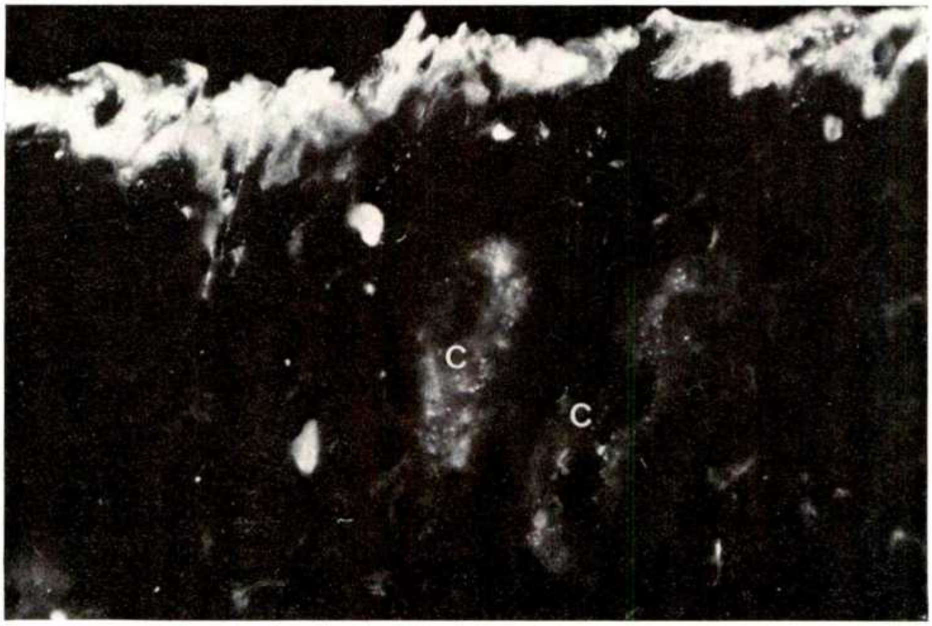

FIGURE 4. Deposition of fibrin in the epidermal basement zone in a LP lesion. The staining does not reveal pathology in the capillaries (C) (Leitz Orthoplan microscope, × 54 oil immersion).

Blood vessels. Staining of vascular structures was never the most impressive feature of LP lesions. In four cases of chronic LP, heavy deposition of fibrin was found in the thickened walls of superficial blood vessels. They were embedded in a massive perivascular meshwork of fibrin. In lesions of shorter duration, the staining was far less conspicuous or absent, even in areas of marked fibrin deposition in the EBZ (Fig. 4). Evidence of occlusion of the lumina was never convincing. The IF staining patterns indicative of the presence of the other plasma factors were essentially the same as those found in healthy skin (Baart de la Faille-Kuyper *et al.*, 1974).

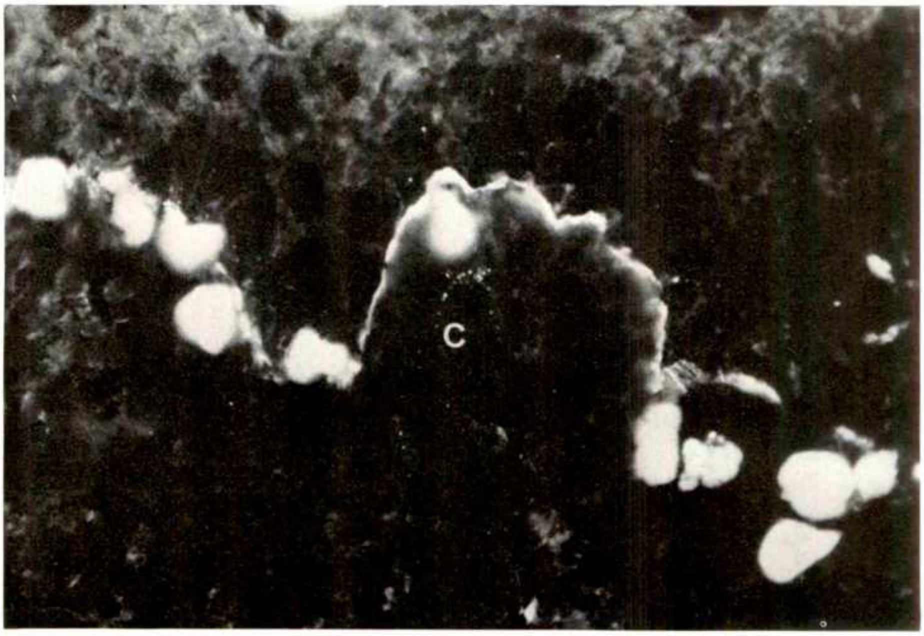

FIGURE 5. Perilesional skin exhibiting the presence of C3,4 in the EBZ and in colloid bodies. The deposition in the EBZ is linear, and granular in the wall of a capillary (C) (Leitz Orthoplan microscope, × 54 oil immersion).

Colloid bodies. CB were demonstrable in variable quantities in all lesions. In smaller quantities they were also detected in perilesional areas (Fig. 5), as well as in three out of five biopsies from clinically uninvolved skin from sites of predilection devoid of microscopical signs of inflammation. They stained brilliantly for IgM, C4, C3c, and C3d, whereas immunofluorescence of fibrin, IgG, IgA, C1q, C3b, C5, and albumin was usually less conspicuous. The fluorescence was either homogeneous or granular (Figs. 2, 3, 4 and 5). Sometimes it was localized around the periphery of the CB.

In conventionally stained duplicate sections of nine lesions, neutrophil and eosinophil leukocytes were observed in the vicinity of CB. Cells containing a brown pigment were often localized in close proximity to CB, sometimes even 'embracing' them. Transillumination of unstained, or IF stained sections revealed that these cells appeared as granular, deep-blue structures. Such cells were also detectable in the upper dermis in perilesional skin, as well as in normal looking skin from sites of predilection.

The epidermis. Speckled staining of epidermal cell nuclei could be demonstrated in lesions of two patients. These cell-bound antibodies were of the IgG class. In one of these patients *in vivo* bound IgG was also demonstrable in the uninvolved skin, mostly in the nuclei of basal cells. Re-examination of the skin of this patient 6 months later yielded identical IF staining results.

Serum antibodies

Antibodies with affinity for nuclear antigens were found in the sera of six patients. The titres ranged from 1/20 to 1/640. They were predominantly of the IgG class. All sera produced speckled staining patterns. Antibodies with affinity for the basement zone of stratified epithelia were not demonstrated.

DISCUSSION

The results of recent studies indicate that the primary aetiological factor(s) in LP damage the epidermis (Thyresson & Moberger, 1957; Sarkany & Gaylarde, 1971; Black & Wilson Jones, 1972). By means of electron microscopy, Ebner & Gebhart (1972) have shown that a fibrillar transformation in the basal epidermal cells results in the formation of colloid bodies. The results of the present study also lend support to the view that the primary pathology in LP affects the epidermis. The earliest pathology we noticed consisted of the presence of colloid bodies and pigment containing macrophages (indicative of pigmentary incontinence) which occurred in early lesions, in perilesional areas, and even in clinically normal skin from sites of predilection.

The concomitant occurrence of C1q, C4, C3-fragments and C5 in or around the colloid bodies indicates that complement has been activated in or on these structures via the classical pathway. How this activation has been achieved, and whether it is the cause or the result of the epidermal pathology, remain to be answered. Several possible explanations present themselves. The concomitant occurrence of immunoglobulins and complement may indicate that the immunoglobulins have played a part in the activation process. If this were true, it must be determined whether the immunoglobulins (either in the role of antibodies or as non-antigen-induced aggregates) are primarily involved in the genesis of colloid bodies, for it is also conceivable that a secondary (auto)immunological mechanism is directed against antigenic debris of degenerated basal cells. The occurrence of anti-nuclear antibody in the serum of six patients, as well as bound *in vivo* in two LP lesions and one uninvolved skin, may be explained on a similar basis. The persistent presence of anti-nuclear antibody in the clinically normal skin of one patient argues against a primary pathogenic role of these antibodies in LP. The significance of anti-EBZ antibodies with regard to the pathogenesis of bullous variants of LP (Baart de la Faille-Kuyper, 1972) also remains to be clarified. These antibodies may be directed against altered EBZ-antigens but may cross-react with normal EBZ-antigens.

Great care should be exercised in interpreting the finding of IgM and of complement components and fragments in the EBZ, as IgM, C3-fragments, and traces of C5 can also be found in the EBZ in the skin of healthy individuals (Baart de la Faille-Kuyper *et al.*, 1974). The differences between these two substrates are mostly quantitative and may result from the pathologically enhanced permeability of the capillaries in LP. This pathology is evidenced by the accumulation of fibrin in the upper dermis.

Apart from the above considerations, it should be remembered that several other pathways exist by which complement may be activated in LP lesions. These include enzymes generated during the interactions of coagulation, fibrinolysis, and kinin systems (Ratnoff & Naff, 1967; Donaldson, 1968), and other proteolytic enzymes. Whatever the mode of action, it seems logical to suggest that biologically active complement fragments or components which are generated during the activation sequence (the chemo-attractants C3b, C5a, and $C\overline{567}$) may explain the accumulation of polymorphonuclear leukocytes in early stages of LP. The presence of fibrin in the upper dermis may contribute to this phenomenon (Barnhart, 1968). Besides attracting leukocytes, fibrin may also be important in determining whether the local inflammatory state is perpetuated.

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The epidermal disorder in lichen planus: an *in vitro* study*

D.G.C.PRESBURY AND R.MARKS

St John's Hospital for Diseases of the Skin, Institute of Dermatology,
Lisle Street, London, W.C.2

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SUMMARY

The epidermal component of lichen planus seems to result from a continuous process of erosion of the basal zone, and repopulation of the 'wounded' area by cells from nearby uninvolved epidermis. In order to investigate these migrating cells further, we have studied tissue slices from forty papules of lichen planus derived from twenty-nine patients. The tissue slices were cultured for periods of 24 or 48 h. Marked migration of fusiform and spindle-shaped cells into the eroded dermo-epidermal zone appeared to have taken place. In general, the changes represented an exaggeration of those usually present *in vivo*. Cytochalasin B, in concentrations of 5 μg/ml or greater in the culture medium, prevented cell migration. This effect, and the fact that autoradiographic studies showed few cells to be synthesizing DNA, suggested that cell migration took place by movement rather than by mitotic activity.

Lichen planus (LP) seems unusual in that, although there is destruction in the basal generative layer, there is at the same time epidermal thickening. A recent study by Marks, Black & Wilson Jones (1973) has produced evidence suggesting that keratinocytes migrate into the LP lesion from nearby uninvolved epidermis, and then repopulate the damaged basal zone in an attempt at healing the 'microwound' due to the erosive LP process. We have tried to learn more about these epidermal changes, by studying *in vitro* tissue slices from LP papules. During this study we have made observations that we believe support the concept that the epidermal changes in LP are a mixture of continuous damage and of attempts at repopulation by migrating epidermal cells.

METHODS

Forty papules of LP lesions from twenty-nine patients were removed at biopsy, and 1 mm vertical slices were prepared using disposable scalpels (Gillette). Portions of each papule, submitted to routine histological examination, confirmed the presence of LP. Three papules from patients over 40 years of age were injected intracutaneously with 10 μCi of tritiated thymidine (Radiochemicals, Amersham, specific activity 22 Ci/mmol) in 0·1 ml of normal saline. The injected sites were removed 1 h after injection, and then cut into 1 mm vertical slices as before.

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* Based on a paper read at the meeting of the European Society for Dermatological Research, Amsterdam, 25 April 1973.

All slices were incubated for 24 h (seventy-nine slices) or for 48 h (twenty-four slices) in Eagle's minimum essential medium. The details of the culture procedure have been given elsewhere (Marks, Bhogal & Dawber, 1972). Tritiated thymidine was added to eight cultures in a concentration of 1 μCi/ml (specific activity 23 Ci/mmol, batch number 23) during the first 4 h of culture, after which the slices were washed several times in cold medium, and then replaced in Petri dishes containing the medium alone. Culture then continued for a further 20 or 44 h (four slices for each time). For the remaining seventy-five cultures incubation continued until 4 h before the end of the 24 or 48 h culture period. At that time tritiated thymidine was added in a concentration of 1 μCi/ml, and exposure continued over the final 4 h. When culture had finished, the eighty-three slices to be studied histologically and autoradiographically were washed in normal saline and fixed in 10% neutral formalin. After dehydration in graded alcohols, they were embedded in paraffin and sectioned at 5 μm. Sections for autoradiography were 'de-paraffinized' and dipped in Ilford nuclear emulsion K2 (Ilford, Nuclear Research, Essex) and exposed at 4°C for periods of 1, 2 and 3 weeks. After exposure they were developed in Kodak D.19 developer. Before inspection, the autoradiographs were stained through the emulsion with haematoxylin and eosin, and mounted in 'Permamount'.

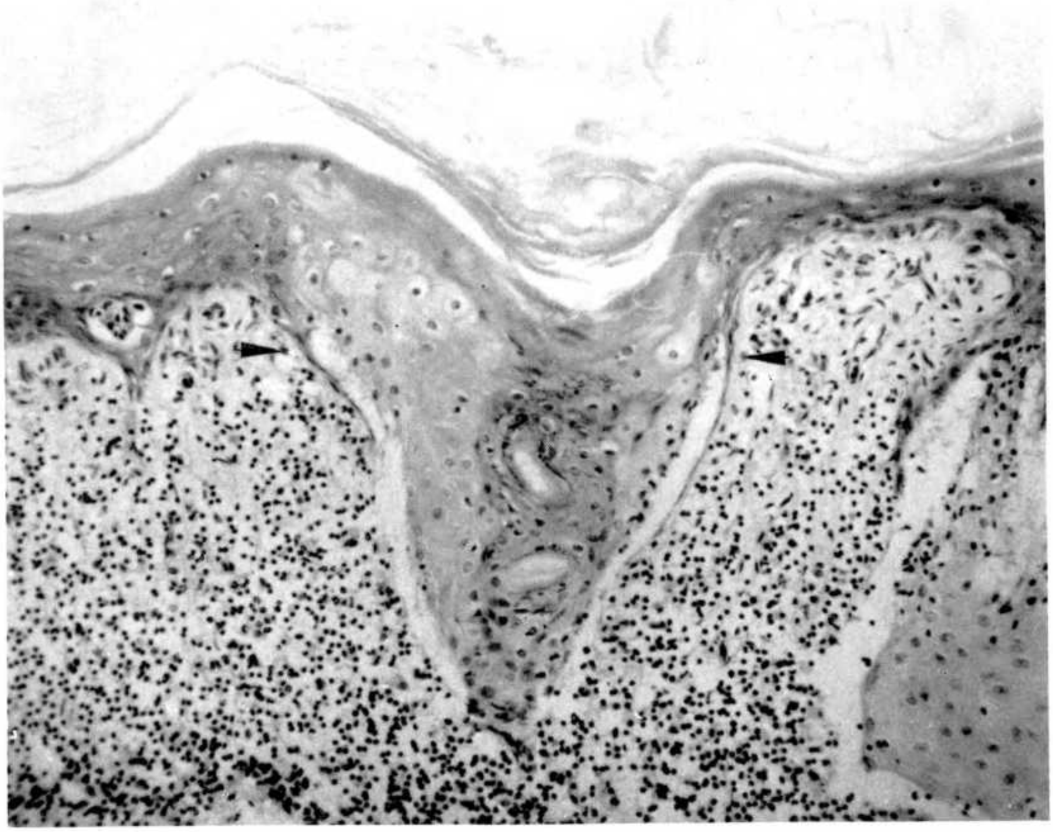

FIGURE 1. 24 h culture of lichen planus tissue. Files of fusiform cells (arrowed) arising from the uninvolved epidermis, line a cleft. There is severe degenerative change of the thickened epidermis (H & E, ×90).

Twenty-four slices in culture were exposed to cytochalasin B in alcoholic solution, added in concentrations of 2 (three slices), 5 (twelve slices), and 10 (nine slices) μg/ml. The final concentrations of ethyl alcohol did not exceed 1%. When cultures containing cytochalasin were set up, parallel cultures containing tissue from the same papules, containing the same concentration of alcohol but no cytochalasin, were also set up.

Enzyme histochemical tests

Nine slices, after 24 h culture, were quenched in liquid nitrogen and sectioned at 10 μm on a Bright's

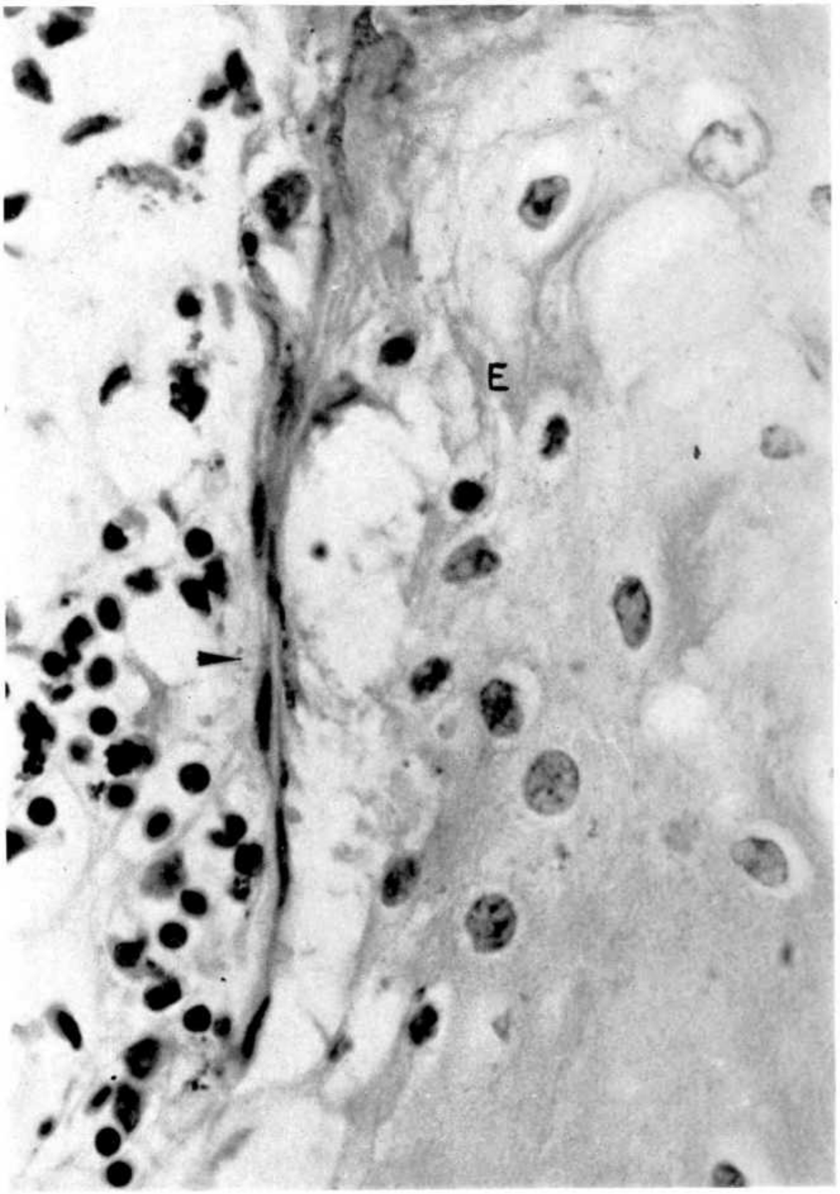

FIGURE 2. Detail from Fig. 1 to show the fusiform epidermal cells (arrowed) and degenerate epidermis (E) (H & E, × 300).

crytostat. The following enzyme histochemical reactions were performed on cryostat-sectioned tissue: (a) lactic dehydrogenase, (b) NADH diaphorase, (c) succinic dehydrogenase (using the nitroblue tetrazolium method outlined in Pearse, 1960).

Electron microscopy

After 24 h in culture, nine slices were cut into 1 mm cubes, fixed in 2·5% glutaraldehyde, washed in cacodylate buffer and dehydrated in graded alcohols before being embedded in Epon. The Epon blocks were sectioned on an LKB ultramicrotome and examined on copper grids in an EMI 6B electron microscope at an accelerating voltage of 60 kV.

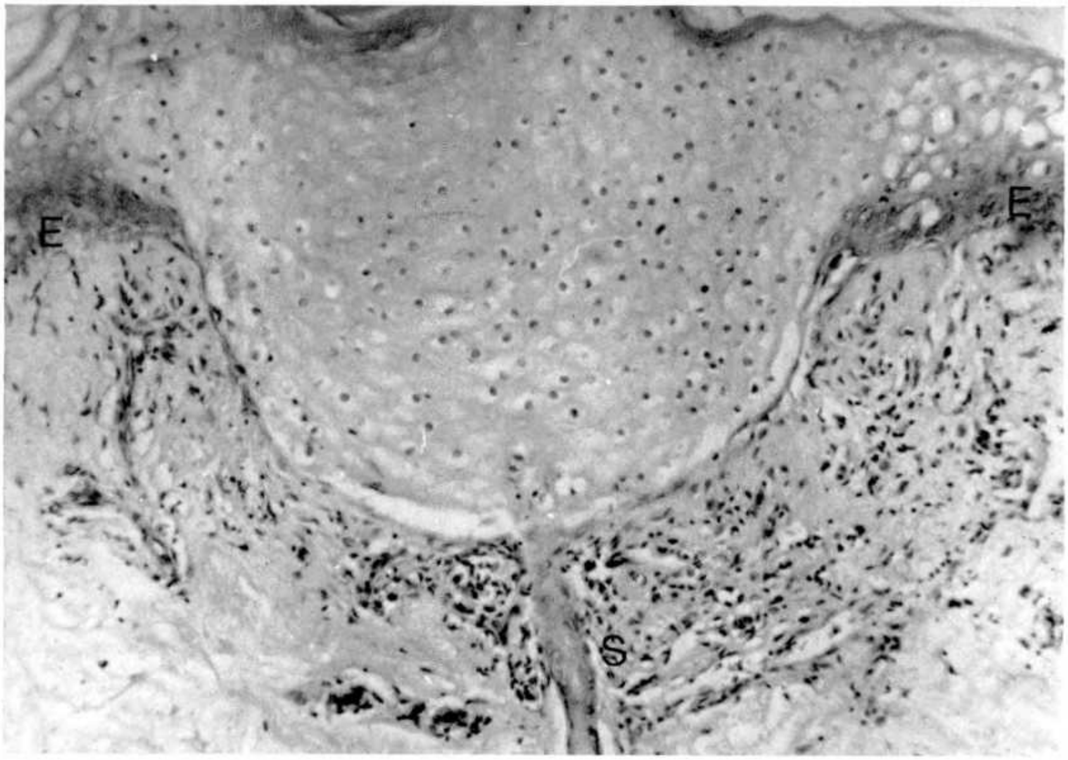

FIGURE 3. Lichen planus tissue cultured for 24 h showing files of fusiform epidermal cells arising from a sweat duct (S) and uninvolved epidermis (E).

RESULTS

Histological observations

The changes seen in the LP papules cultured *in vitro* were distinctive and seemed to represent an exaggeration of those seen in freshly removed lesions. The most dramatic and consistent change was the appearance of columns and files of spindle-shaped or fusiform, basophilic cells streaming into the affected dermo-epidermal zone (Figs. 1 and 2). These columns were continuous with adjacent uninvolved epidermis, or with the epithelium of nearby sweat ducts (Fig. 3). The cells of the upper epidermis were quite swollen, pallid, and vacuolated (Fig. 2). Degeneration of the basal zone was obvious, with long areas of separation at the dermo-epidermal junction, emphasizing the space or potential space found *in vivo*, termed the Max Joseph cleft. The degeneration in the upper epidermis was a striking and characteristic feature. We have not seen this degree of degenerative change after culture of normal skin or of the lesions of many other dermatoses after 24 or 48 h.

Autoradiographic observations

None of the autoradiographic preparations showed large numbers of labelled epidermal cells. Most of the labelled epidermal cells were of the spindle-cell type and were present in groups and clusters.

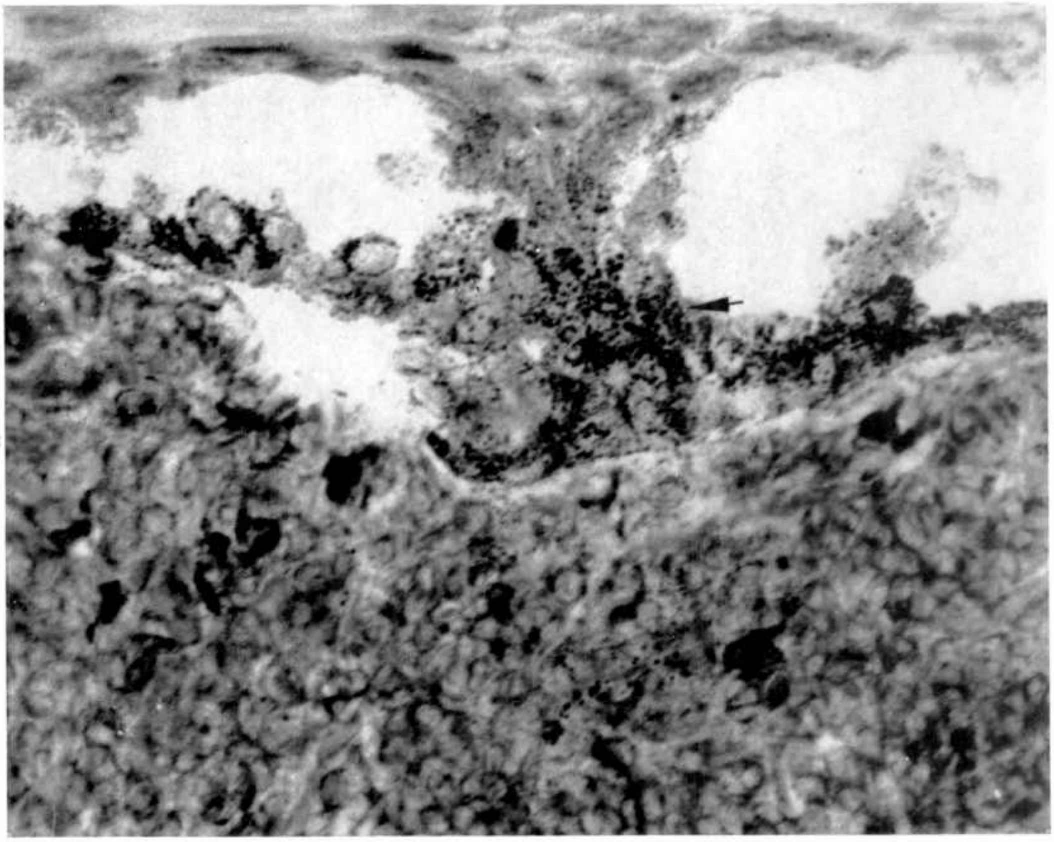

FIGURE 4. Lactic dehydrogenase reaction in lichen planus tissue after 24 h of culture. From one point in the largely non-reacting epidermis two columns of cells are seen (arrowed) which show many formazan granules within them. The infiltrate in the dermis shows some weak reactivity (cryostat section, lactic dehydrogenase reaction, × 300).

The impression was that there was a greater proportion of labelled cells in those tissues that had been injected with tritiated thymidine prior to removal and incubation, than in those exposed to tritiated thymidine during culture. Because of the disruption and irregularity of the epidermis, it was not possible to produce a formal labelling index.

Enzyme histochemical observations

The slices examined enzyme histochemically showed deposition of formazan reaction product in the migrating cells, with very little activity in the rest of the epidermis and in the dermal infiltrate. The reactions for NADH diaphorase and lactic dehydrogenase were strongly positive (Fig. 4), but the succinic dehydrogenase reaction was only weakly so.

Cytochalasin studies

Twelve tissue slices exposed to 5 μg/ml, and nine slices exposed to 10 μg/ml concentrations of cytochalasin B, showed no columns or files of spindle-shaped migrating cells. Three slices exposed to 2 μg/ml showed the same changes (including marked migration) as the slices cultured without additions. Control cultures made at the same time, and containing the same quantity of alcohol as the cytochalasin cultures, showed the same changes including migration as did the other cultures. There were other unexpected findings in the slices exposed to 5 and 10 μg/ml concentrations of cytochalasin B. Suprabasal clefts formed, and the epidermal cells of that region resembled the acantholytic cells with disordered arrangement seen in pemphigus vulgaris. They appeared rounded and had only tenuous contact with neighbouring cells (Fig. 5).

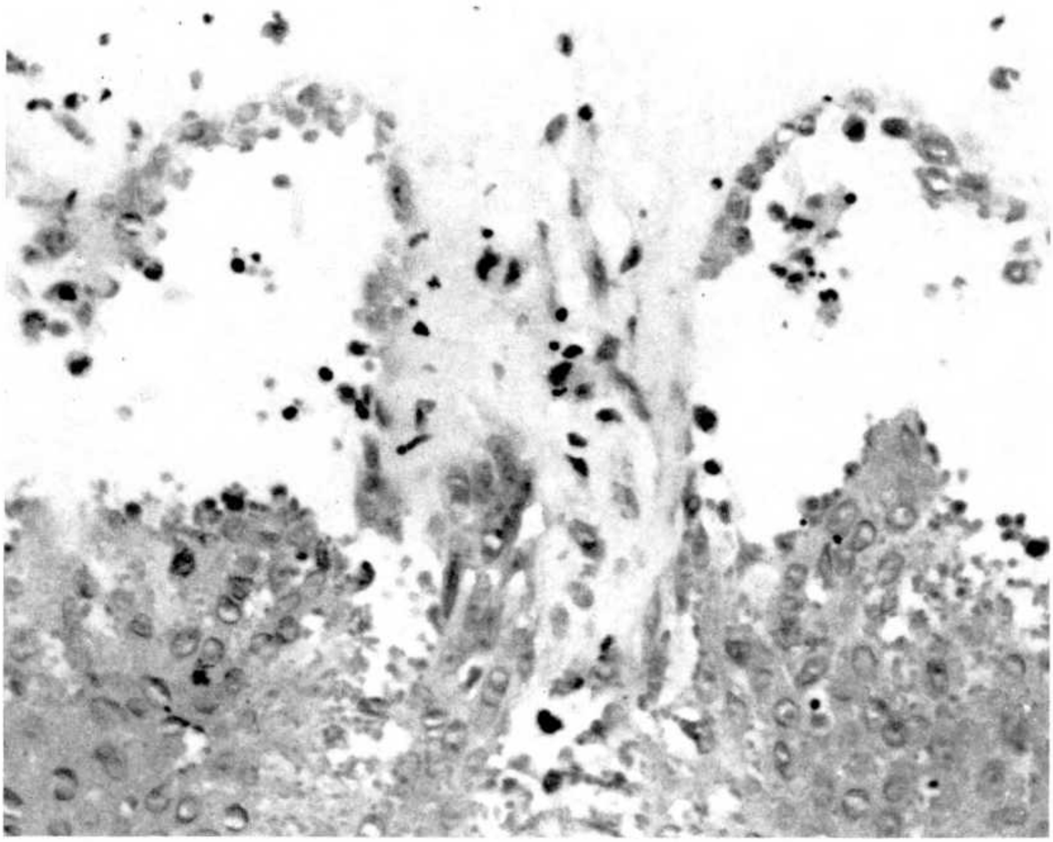

FIGURE 5. The effects of cytochalasin (5 μg/ml) on lichen planus tissue after 24 h in culture. There is a slight resemblance to the histological appearance of pemphigus with a suprabasilar cleft and rounded 'acantholytic' cells (H & E, × 220).

Ultrastructural observations

Migrating cells were identified on electronmicrographs at the base of the epidermis, close to inflammatory cells. No intervening basal lamina was found between these cells and the inflammatory infiltrate. The cells were rich in ribosomes and rough endoplasmic reticulum. They also contained melanin granules, but keratinosomes and keratohyaline granules were not seen. In addition to filamentous bundles, identified as tonofilaments, there were in some cells other fine filaments in bundles and whorls, that we believe represented microfilaments. These migrating cells possessed long microvilli and few desmosomal contacts.

DISCUSSION

Epidermal cell migration was a striking feature of LP tissue *in vitro*. In normal skin in culture, some epidermal cell migration occurs at the ends of the skin explant in a process termed epiboly, but this does not resemble in its location or extent the changes that we now describe in LP. Active epidermal cell movement seems to have taken place in the material from LP, despite the absence of serum, which appears to be necessary to promote epiboly in normal skin *in vitro* (Marks *et al.*, 1972; Levine, 1972).

Cytochalasin B (a compound derived from fungal metabolites) inhibits cell movement whilst having no effect on mitotic activity (Carter, 1967; Wessels *et al.*, 1971). It has been suggested that its mode of action is one of disruption of cytoplasmic microfilaments. Cytochalasin has been shown to prevent epidermal cell movement in culture (Wikswo & Szabo, 1972; Constable, Cruickshank & Mann, 1972; Nishikawa & Marks, 1973). This study has shown also that cytochalasin B at a concentration of 5 μg/ml inhibits cell migration in the epidermis. The combination of this effect of cytochalasin, and the observation that relatively few of the epidermal cells showed labelling after exposure to tritiated thymidine in the autoradiographs, supports the concept that cell movement rather than mitotic activity is the main motive force for migration *in vitro* during the first few hours.

Our enzyme histochemical studies showed that the migrating cells were highly active metabolically, and that the pattern of reaction observed was similar to that of migrating cells from normal skin explants in culture (Marks *et al.*, 1972).

Electron microscopy identified the small fusiform cells as epidermal in origin. They contained tonofilaments and had many of the characteristics of basal epidermal cells (Breathnach, 1971). We believe that a system of microfilaments could be seen in some of these cells.

In conclusion, cultured tissue from the papule of LP shows an exaggeration of those histological features found in life. The spindle-shaped cells seen in cultures have been identified as migratory keratinocytes. They arise from uninvolved areas of the epidermis or from sweat coils at the dermo-epidermal junction. The evidence from our previous autoradiographic investigations on LP papules *in vivo*, and the present *in vitro* study, suggests that the thickened epidermis and the eroded saw-toothed basal region in the LP papule result from a mixture of continuous damage to the basal zone, and persistent attempts at repopulation of the area by migrating keratinocytes.

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Oral florid papillomatosis in the course of lichen planus

FELIKS WASIK, ALEKSANDRA WOYTON AND IRENA BIRECKA

Department of Dermatology (Head: Professor J. Kubicz), Wroclaw, Medical Academy, Poland

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SUMMARY

The causal relationship between oral florid papillomatosis and lichen planus is discussed in the light of the literature and a case report.

Rock & Fisher (1960) were the first to introduce the concept 'oral florid papillomatosis' (OFP) to describe chronic hypertrophic pseudo-neoplastic changes in the oral and pharyngeal mucosae. This multiple or confluent form of squamous cell papilloma tended to recur after surgery, and revealed focal atypia but without fulfilling any criteria for malignancy. In the three cases these authors described, metastatic or truly invasive growth was not noted in any instance, and cellular atypia was always confined to the basal cells. Knossew (1966) suggested the term 'papillomatous granulomatosis', and Barnett & Hyman (1968) 'oral florid verrucosis'. However, in 1958 Scheicher-Gottron had described the same condition under the term 'papillomatosis mucosae carcinoides', thinking it to be the oral mucosal counterpart of Gottron's 'papillomatosis cutis carcinoides'. Verrucose and cauliflower-like growths persisted on the tongue for 17 years in a 59-year-old patient known to have had lichen planus for 40 years on the legs and scrotum. The oral lesions were diagnosed clinically as a verrucose squamous epithelioma on a background of lichen planus. The histological appearance was that of an extensive acanthosis and papillomatosis without cellular atypia or other features of malignancy.

Moldenhauer & Putzke (1973) identified fourteen cases of OFP in the literature, and their own case, together with that described by Thormann & Sönnichsen (1972), brought the number of published cases to sixteen; the following case is the seventeenth.

CASE REPORT

A man aged 62 was admitted to the Dermatological Clinic of Wroclaw Medical Academy in April 1972. There was a history of hypertrophic lichen planus affecting the left leg and of oral lichen planus for the previous 4 years. He had smoked 10 cigarettes a day for many years. While the lichen planus was under treatment, a verrucose proliferative growth developed on the left buccal mucosa.

On examination there were extensive palm-sized verrucose lichen planus lesions on the extensor aspect of the left leg (Fig. 1). The diagnosis was confirmed histologically. The buccal mucosa on both

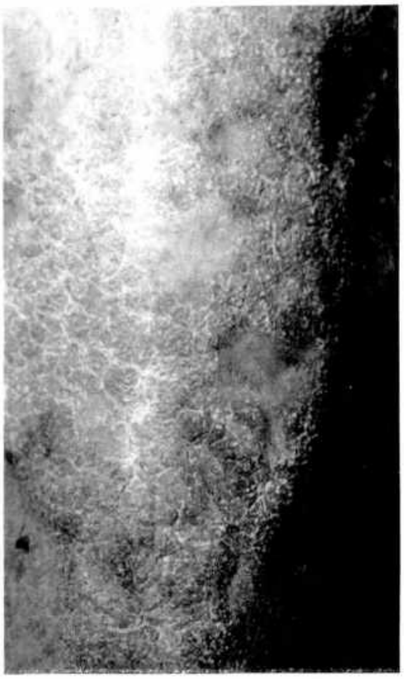

FIGURE 1. Hypertrophic lichen planus of the left leg.

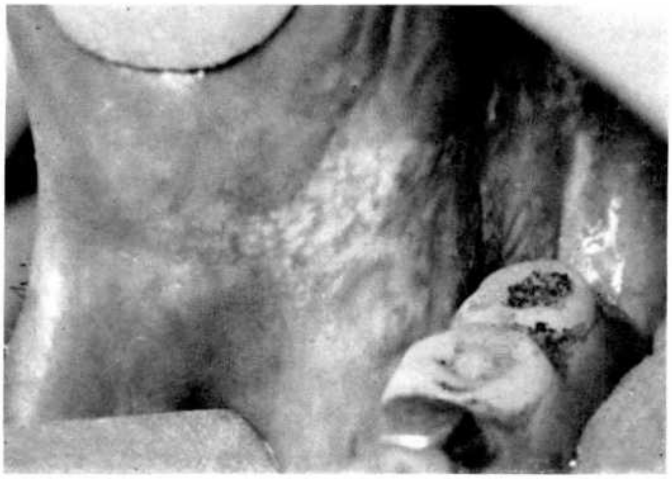

FIGURE 2. Lichen planus of the buccal mucosa.

sides featured a confluent papular eruption on a slightly erythematous mucosa (Fig. 2). Towards the left commissure the mucosa presented an irregular shaped cauliflower-like proliferating tumour (Fig. 3). Its surface was dry and irregular, with deep fissures that merged posteriorly into the confluent pattern of lichen planus similar to that on the other side of the mouth. A clinical diagnosis of a verrucose squamous cell epithelioma associated with lichen planus was made. The regional lymph nodes were not enlarged. A full upper and a partial lower denture were worn.

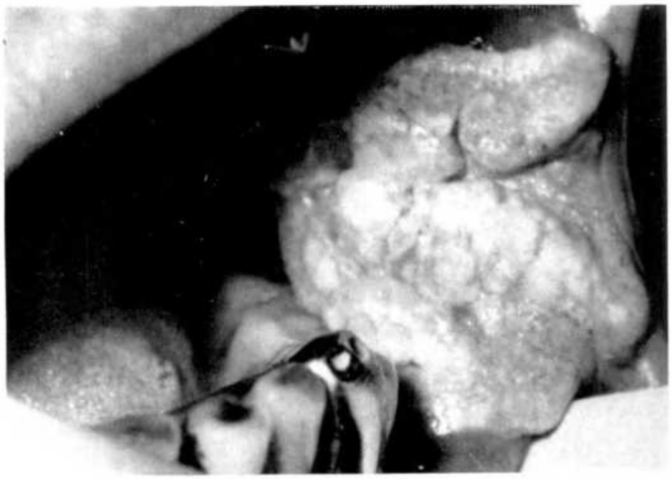

FIGURE 3. Proliferating tumour of the buccal mucosa.

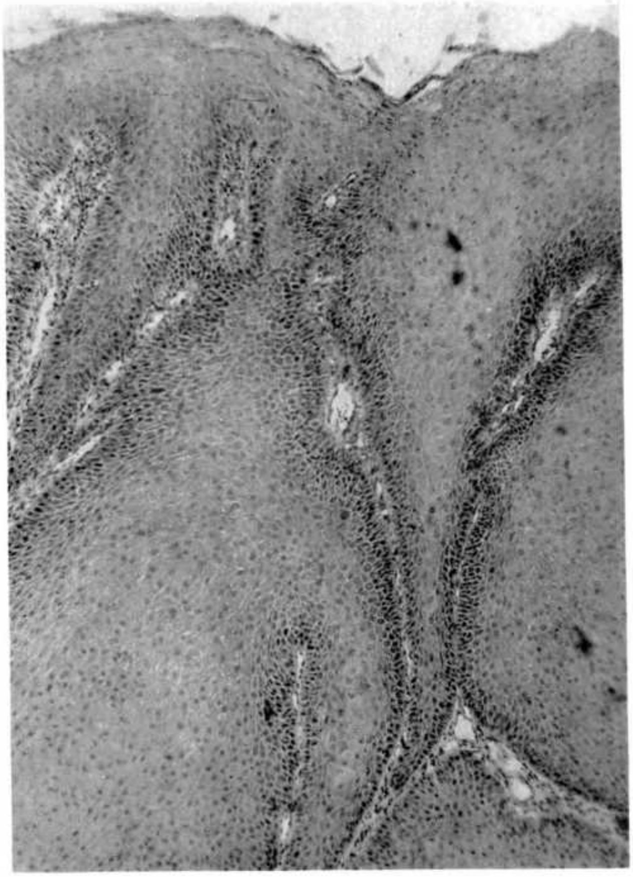

FIGURE 4. Buccal mucosa: epithelial hyperplasia with broad epithelial ridges.

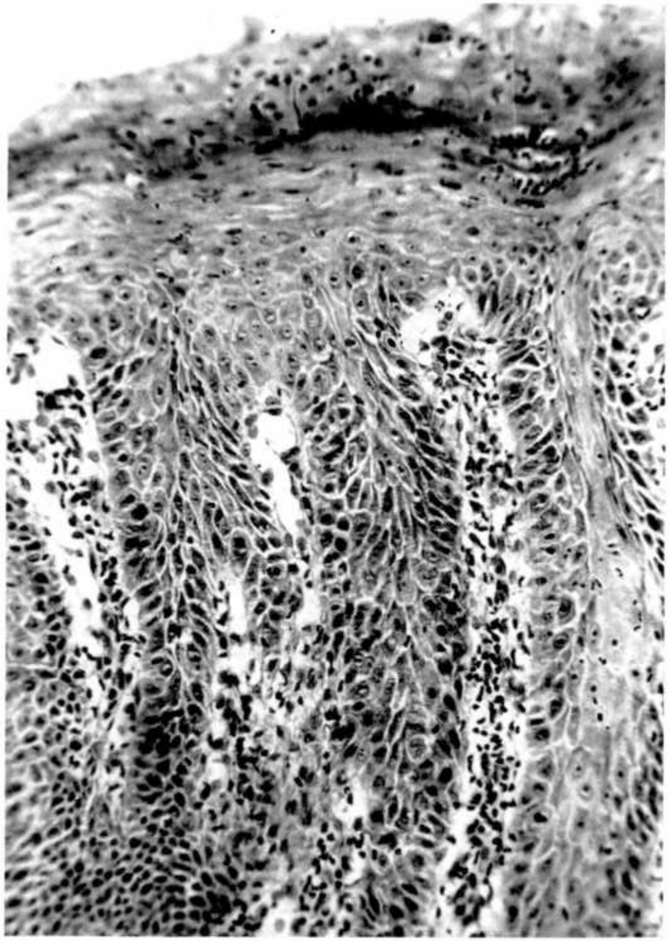

FIGURE 5. Buccal mucosa: elongated epithelial ridges.

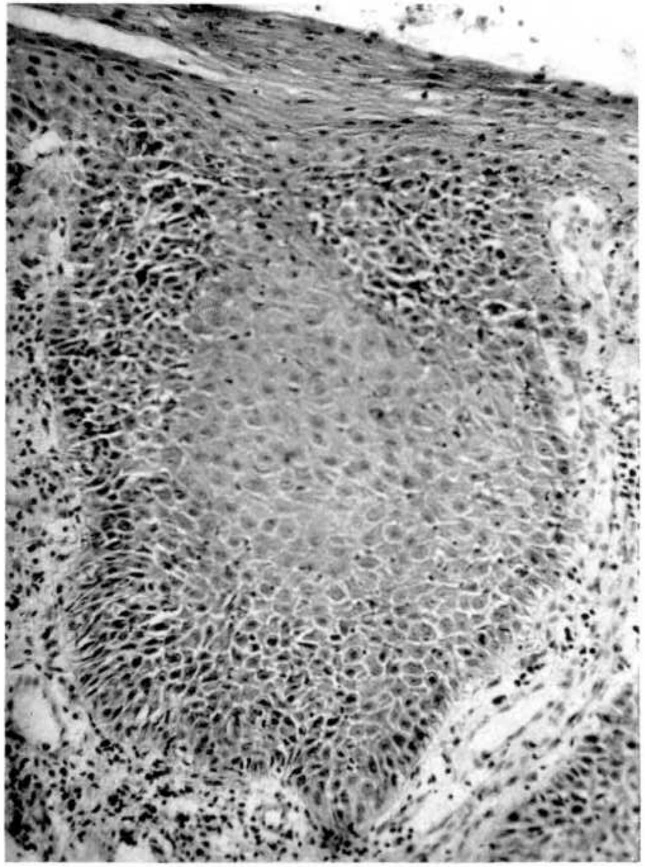

FIGURE 6. Buccal mucosa: the basal layer is clearly defined. There are no abnormal mitotic figures.

Biopsies of the oral and skin lesions were carried out. All the features depicted in oral florid papillomatosis were found in the tumour affecting the left buccal mucosa. There were gross parakeratosis and acanthosis without a granular layer. The hyperplastic epithelial ridges were sometimes 'barrel-shaped' (Fig. 4) and sometimes appeared as thin elongated 'icicles' (Fig. 5). No atypia or abnormal mitotic figures were present, and the basal layer was always intact and well defined (Fig. 6). The adjacent corium featured diffuse infiltration with chronic inflammatory cells. The mucosa adjacent to the tumour, which clinically had appeared to be lichen planus, showed in a mild form some of the features of oral florid papillomatosis.

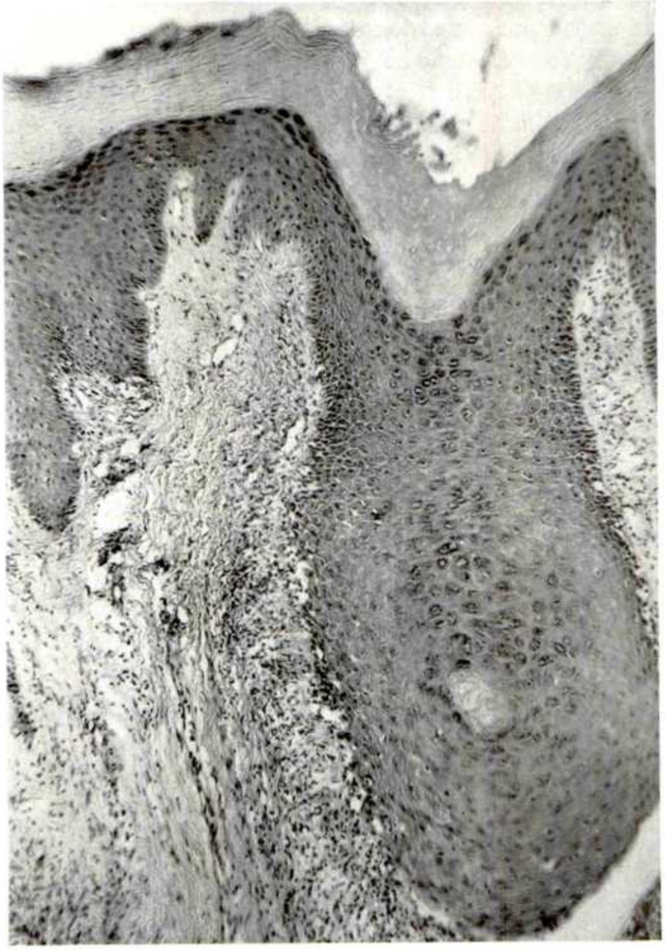

FIGURE 7. Lower leg: histological features of hypertropic lichen planus.

The histological examination of the skin biopsy from the left leg featured hyperkeratosis, acanthosis and an increase in the granular layer. Adjacent to the deep epithelial ridges there was a band-like infiltrate of lymphocytes, and liquefaction degeneration of the basal cells (Fig. 7).

Other findings included the presence of *Candida albicans* on the surface of the oral lesions, normal glucose tolerance tests and negative serological tests for syphilis.

In the absence of any malignant features in the biopsy specimen of the oral tumour, it was excised by diathermy. Seven months after treatment the mucosa at the site of the former tumour still showed the original lichen planus but no recurrence of the tumour.

DISCUSSION

The present case report, together with the literature, show oral florid papillomatosis to be a distinct clinical and histological entity, with a clear cut morphology, a chronic course and a characteristic histology. The lesions may be solitary or multifocal, involving the buccal, less commonly the lingual, and only exceptionally the pharyngeal mucosa. The latter was involved in a case reported by Rock & Fisher (1960). The following conditions are considered in the clinical differential diagnosis: squamous cell epithelioma, condylomata acuminta, deep mycotic infection, and syphilis. The presence of *C. albicans* is thought to be of secondary and not of aetiological significance.

Parakeratosis or orthokeratosis with marked acanthosis and papillomatosis with a well defined epithelial–dermal junction are constant findings. The stratum granulosum is invariably absent.

Samitz, Ackerman & Lantis (1967), Samitz & Weinberg (1969), Kanee (1969) and Degos *et al.* (1970) regard OFP as a premalignant lesion, eventually turning into a verrucose squamous cell epithelioma of low-grade malignancy.

Differing views concerning OFP are presented in the literature. The majority of authors regard the entity as a peculiar precancerous state, following a prolonged course and without lymph gland involvement. Others regard the lesion as a benign neoplasm similar to a viral papillomatosis, although no confirmation of the viral aetiology has been forthcoming. Others regard OFP as a relatively benign and special variety of squamous cell epithelioma. Among the predisposing factors that have been postulated are acute trauma, chronic irritation, smoking and diabetes. Furthermore, there are two cases in the literature, those of Scheicher-Gottron and of Moldenhauer & Putzke, in addition to the present case, in which there is an undoubted association with lichen planus.

All workers agree in stressing the lack of any effective therapeutic procedure; radical excision of the tumour would seem to be the treatment of choice. The prognosis as to a complete cure is unfavourable, with recurrences likely, and even the possibility of true malignancy supervening. The patients are often elderly, and normal life expectancy does not allow very chronic OFP to complete its full natural development.

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Scanning electron microscopy of the normal epidermis and the epidermis *in vitro*

R.MARKS AND B.BHOGAL

Department of Experimental Pathology, Institute of Dermatology,
Lisle Street, Leicester Square, London WC2H 7BJ

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SUMMARY

A simplified technique has been used to examine the epidermis in five skin biopsies and in eight cultured skin specimens by scanning electron microscopy. Paraffin or cryostat sections of the tissue were cut and prepared according to routine histological methods and then given an electrically conducting coating before examination in the scanning electron microscope.

The epidermis was easily identified and a particularly good view was obtained of intercellular bridges at high magnifications. Intra-epidermal and basal layer dendritic cells were also easily located and their relationships to surrounding keratinocytes by long intercellular processes are described.

The scanning electron microscopic appearance of epidermal cells in cultured explants of skin showed a strong resemblance to other cells and tissues in culture and also demonstrated the presence of a unicellular or subcellular membrane at the leading edge similar in appearance to the undulating membrane of fibroblasts in culture. It is stressed that the method is of particular use in the examination of intercellular relationships within the epidermis and in the inspection of histological material at higher magnifications than are possible by light microscopy.

Light microscopy of routinely fixed and prepared histological sections of skin has many severe limitations. An obvious example is the difficulty that may arise if high magnification is required, because of the limited resolution possible using ordinary optical systems. There is also a very limited depth of focus with light microscopy which makes proper appreciation of tissue interrelationships extremely difficult. The object of this communication is to describe our experience with a simple technique for inspecting the epidermis in histological sections at high magnification by scanning electron microscopy (SEM) and to point out the potential usefulness of this procedure.

MATERIALS AND METHODS

Five biopsies of normal human skin obtained from volunteers, who had given their informed consent, were examined in this investigation. In addition, four fragments of human skin, cultured *in vitro* for 2 days according to the methods described by Marks, Bhogal & Dawber (1972), were routinely prepared for histological examination after formalin fixation. 5 μm sections were cut from these nine paraffin blocks and placed on glass microscope slides as for routine histological examination. These sections were then 'deparaffinized' in xylene. Each section was then mounted on an aluminium SEM stub by cutting out the portion of the glass slide bearing the section and sticking it to the stub with an adhesive (Permabond). Adjoining sections to those prepared for SEM examination were stained with haematoxylin and eosin and mounted for routine light microscope examination.

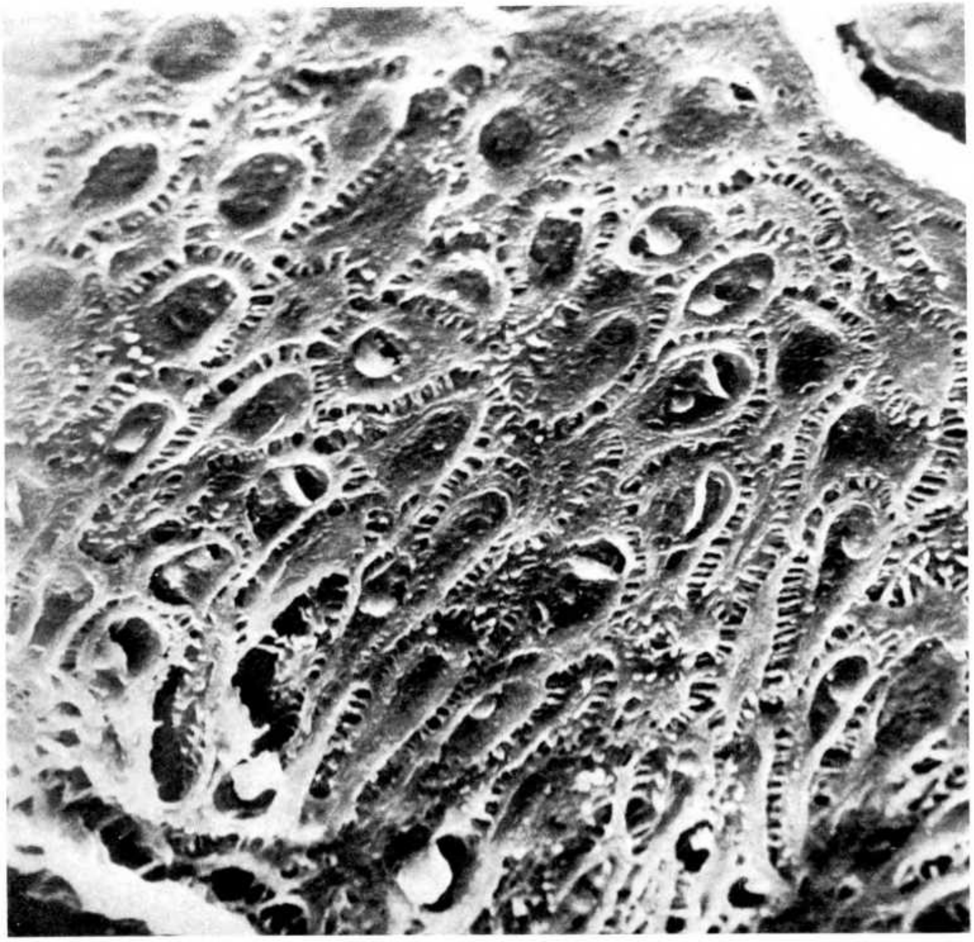

FIGURE 1a. These scanning electron micrographs of formalin fixed sections of epidermis show intercellular bridges particularly well. Some of the bridges have a slight fusiform thickening midway between adjacent cells (a, × 800; b, × 4000).

Four other skin cultures were quenched in liquid nitrogen and sections were cut on a Bright's cryostat at 10 μm. These sections were then dehydrated in graded alcohols and acetone (three changes) and then stuck to SEM stubs as with the formalin fixed specimens. The specimens were then coated with a thin layer of carbon or gold–palladium in an Edward's coating unit. The coated specimens were examined in either a Cambridge Stereoscan SEM (2a) or a Cambridge 600 SEM at operating voltages of 7·5–25 kV.

RESULTS

Deparaffinized sections

The epidermis was easily located at low magnifications; at magnifications of more than 200, intercellular bridges became visible (Fig. 1, a and b). At intervals within the epidermis including the basal layer, there were clear spaces within which were shrunken cells which did not seem to possess proper intercellular bridges but did have long and tenuous dendritic types of processes (Fig. 2). These spaces and their shrunken contents were identified as 'clear' cells on adjoining sections examined by light microscopy. The basal type of 'clear' cell is seen in Fig. 3. The dendritic processes from the basal clear cells sometimes seemed to indent the neighbouring keratinocytes in the same way that a probing finger indents a toy rubber balloon. The surface of the keratinocytes appeared to have a large central oval indentation which, in turn, in some cells seemed to possess a smaller rounded elevation corresponding to the usual location of the nucleus. The intercellular bridges were cylindrical in shape

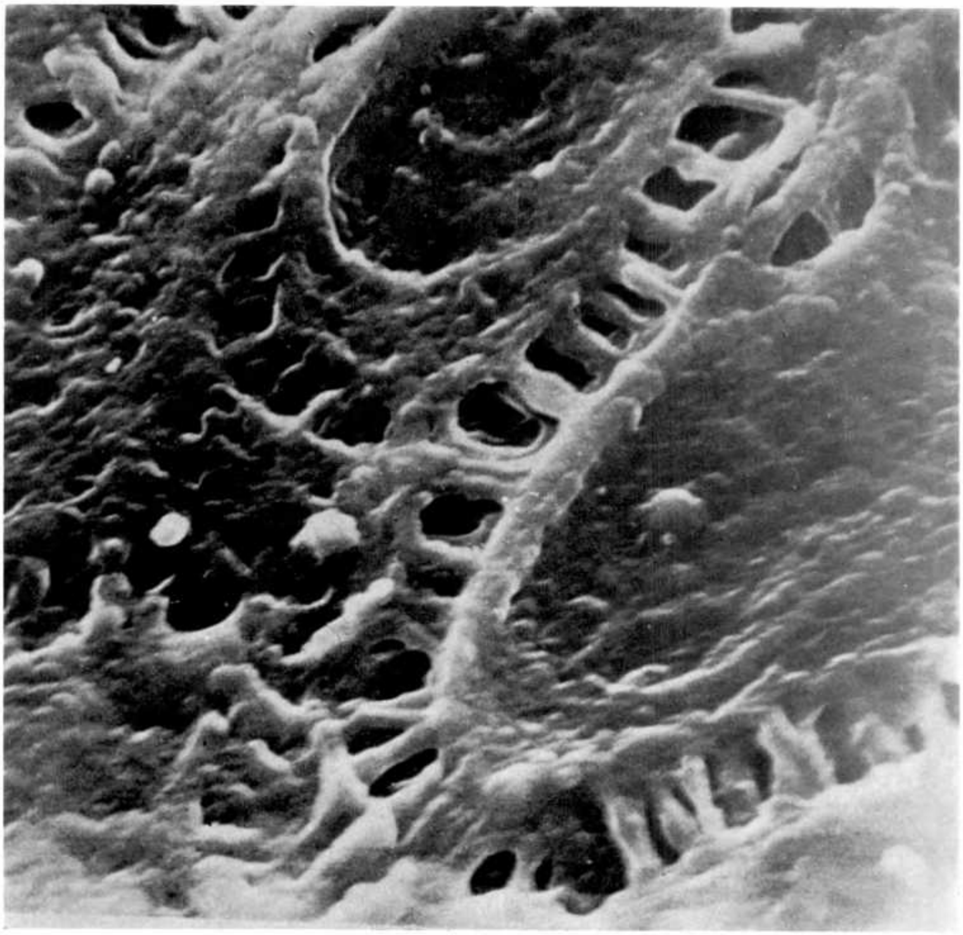

FIGURE 1b. See Fig 1a.

but often had rounded dilatations midway between the neighbouring cells. No special structure or membrane was visible at the basal regions. The cultured specimens all showed epidermal cell migration or epiboly (Medawar, 1948; Sarkany, Grice & Caron, 1965; Marks *et al.*, 1972). Especial attention was devoted to the migrating epidermal cells. The free edge of the migrating sheet of cells seemed to taper to a thin uni- or sub-cellular prolongation reminiscent of the undulating membrane seen at the free edge of monolayer cultures of fibroblasts (Fig. 4). The migrating cells appeared to be fusiform in shape and intercellular contacts were not easily seen. The epidermal cells within the explant were identical with those seen in the biopsies but were more widely separated.

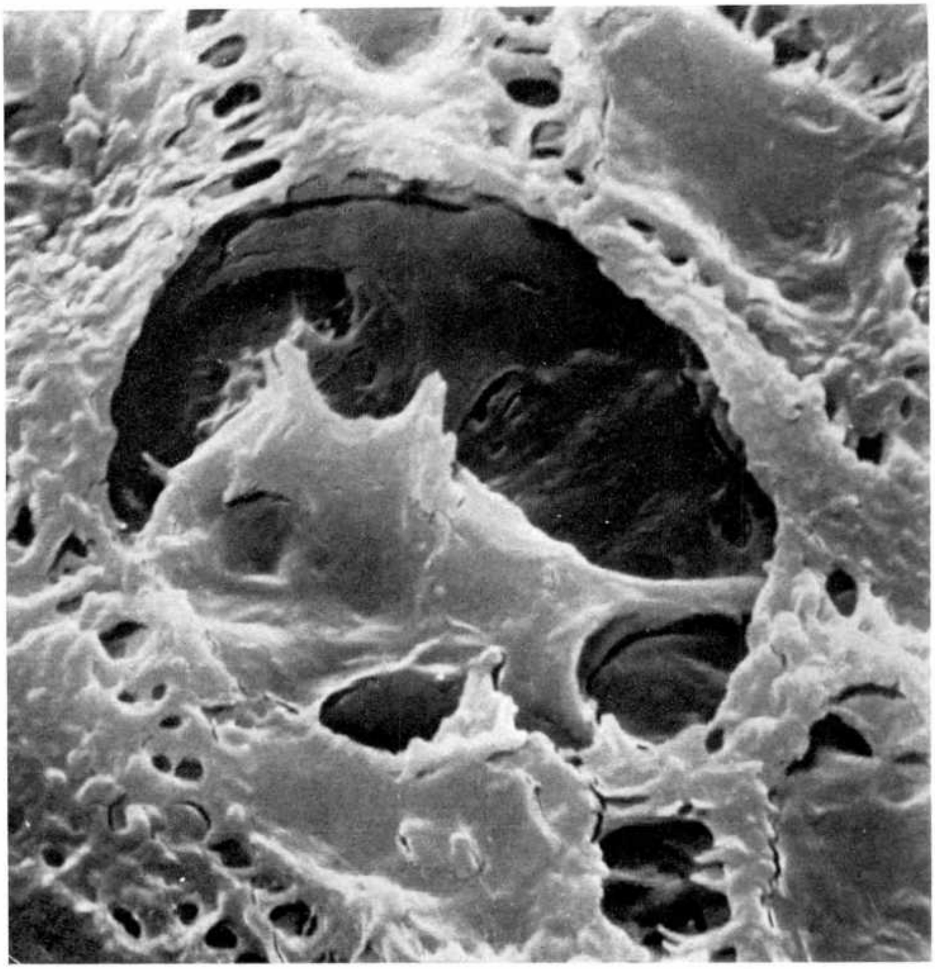

FIGURE 2. Intra-epidermal clear cell as seen with the SEM (× 4640).

Cryostat sections of cultured skin

The surfaces of the epidermal cells in the bodies of the explants were strikingly different from those in the formalin fixed material. The cells did not appear flattened and the site of the nucleus was not

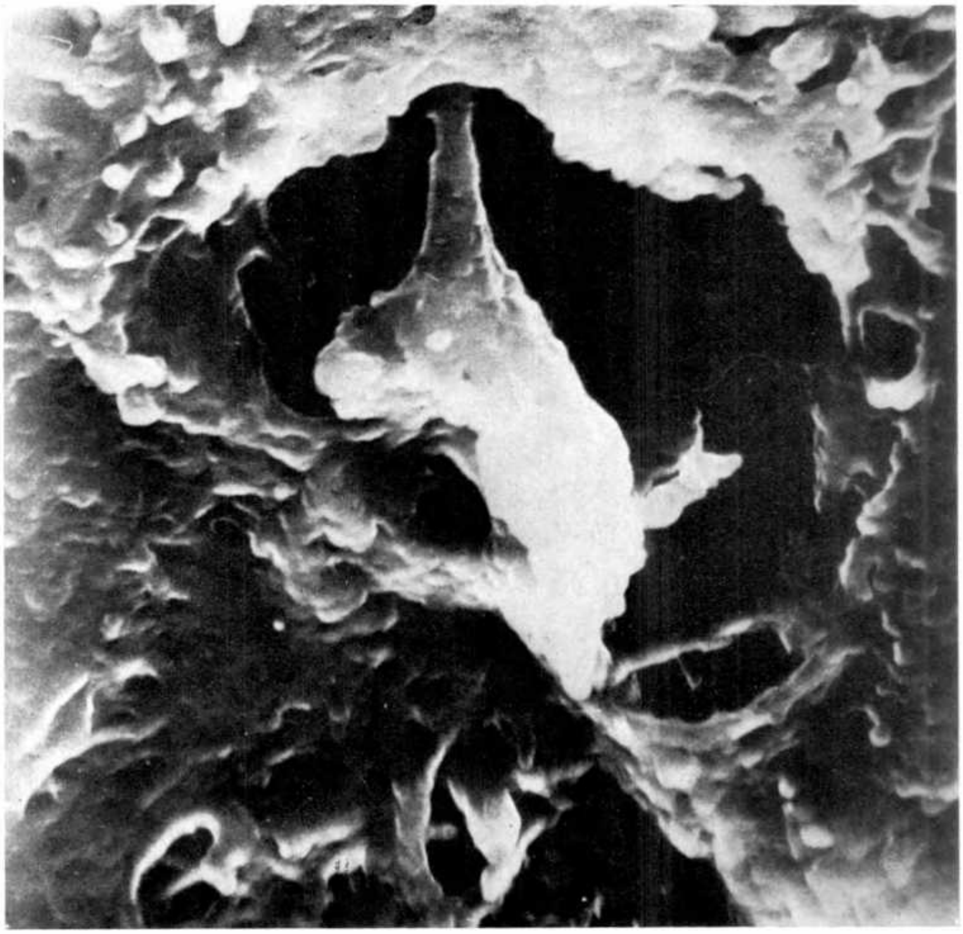

FIGURE 3. Basal layer clear cell as seen with the SEM. There is a long dendritic process apparently penetrating a neighbouring epidermal cell (× 4000).

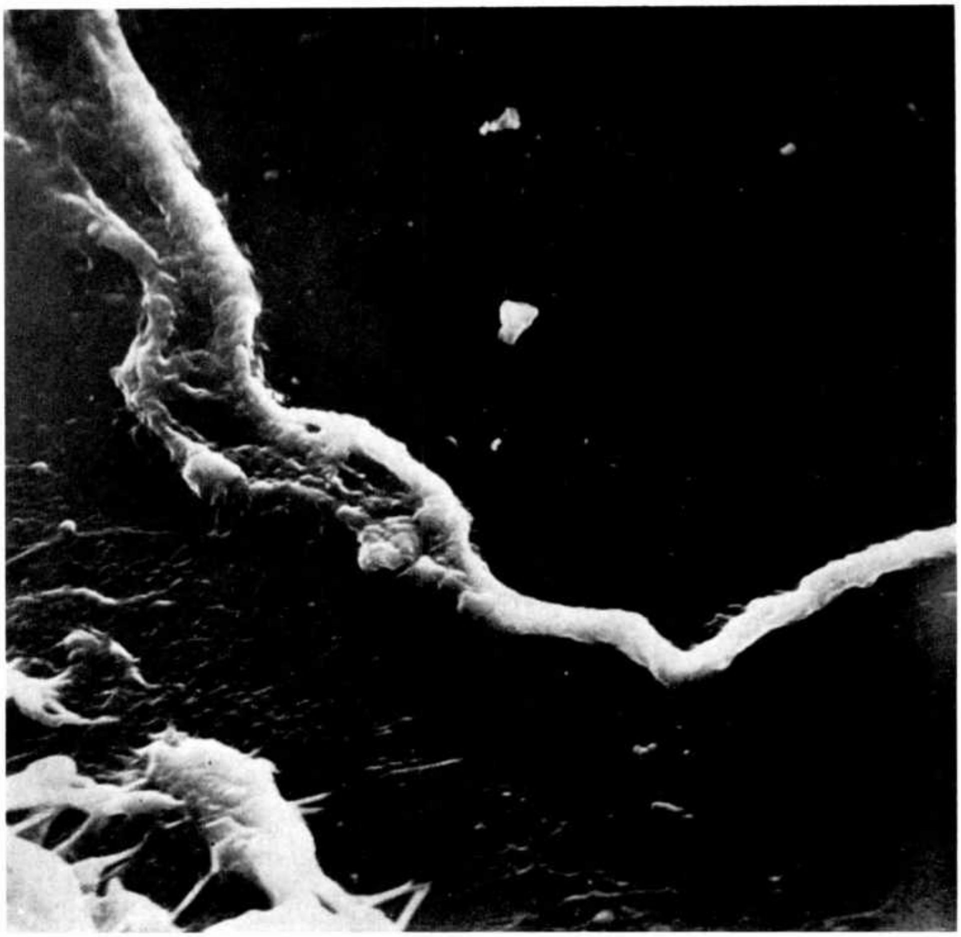

FIGURE 4. Leading edge of migrating cells showing thin uni- or sub-cellular prolongation (× 1600).

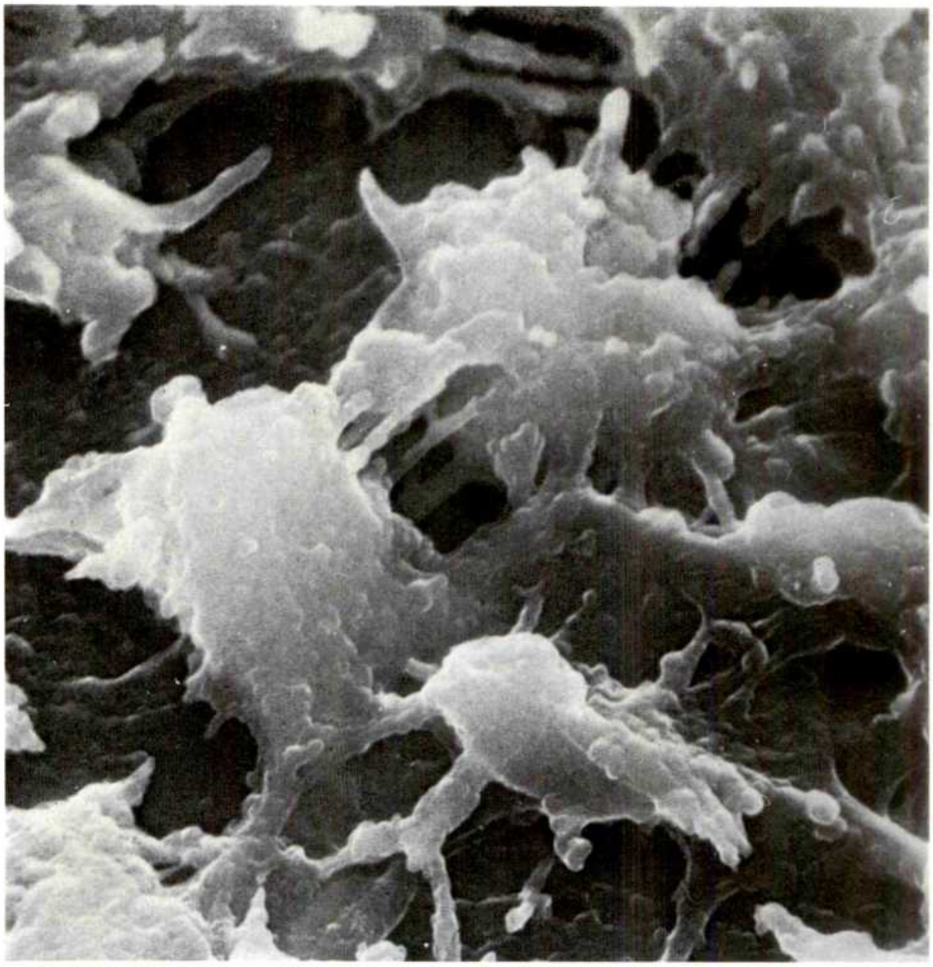

FIGURE 5. Scanning electron micrograph of area of migrating epidermis in a 2-day organ culture of skin. Note long intercellular contacts (× 4800).

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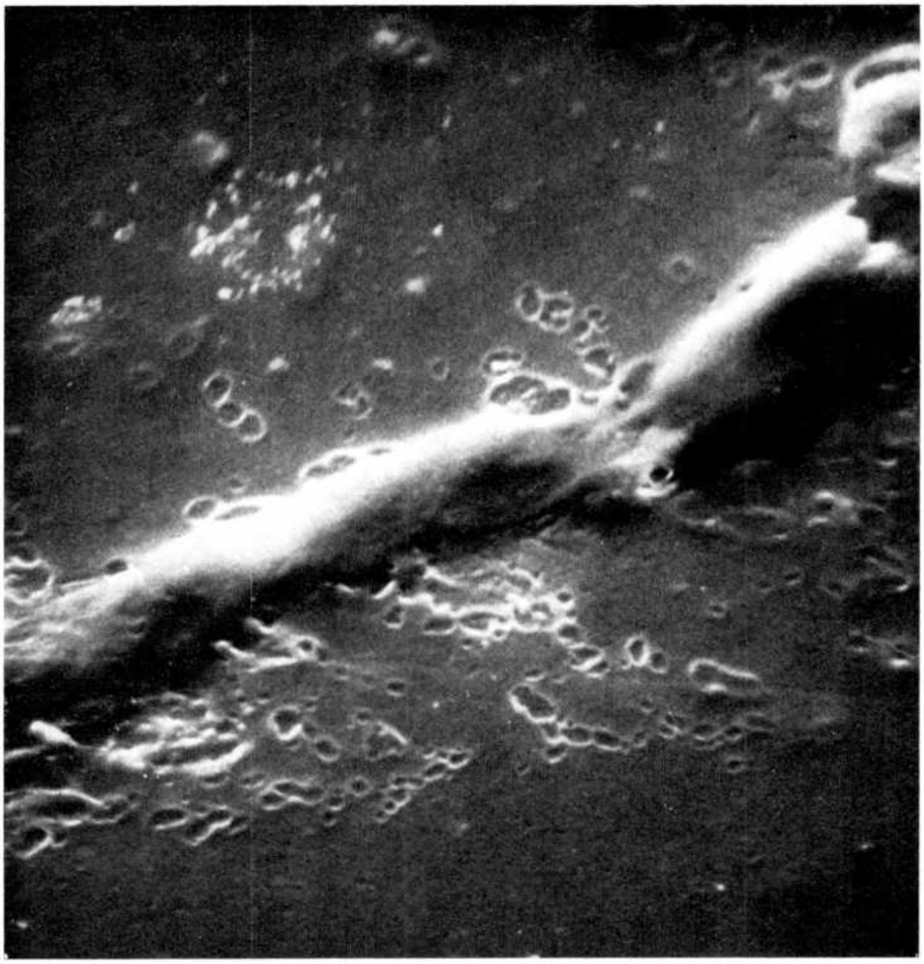

FIGURE 6. Migrating epidermal cells seen with SEM (×4640).

easily seen. There were many long intercellular projections, some of which joined with similar projections on neighbouring cells and probably represented intercellular bridges (Fig. 5). Intercellular connections were not easily seen in the migrating epidermis. In Fig. 6 can be seen a section of a unicellular sheet of cells in which intercellular connections are not readily visible. An oval area can be made out in the body of these migrating fusiform cells which may be the site of the nucleus.

DISCUSSION

McDonald, Pease & Hayes (1967) seem to have been the first to examine sections of formalin fixed tissue in the SEM. They inspected sections of rabbit brain in this way and concluded that the method had application for other tissues. The first to examine human skin in this way appear to have been Tosti *et al.* (1969). They examined sections of 'hyperplastic' epidermis, and described the intercellular bridges and the surface structure of individual keratinocytes in detail. More recently the technique has been used by other groups (Millington & Brown, 1970; Carr, 1970; Wong & Vickers, 1972), who mostly have paid attention to the structure of the dermis and the adnexae. We cannot trace any reports in which the SEM appearances of the epidermal dendritic cells have been described using this technique. Papa & Farber (1971) did, however, see melanocytes when examining the underside of the epidermis after outlining the pigment cells using the DOPA reaction.

Active movement of epidermal cells takes place during the early stages of wound healing and in the process of migration seen at the edges of explants of skin *in vitro*. There have been several SEM studies of cells and tissues in culture; for example that of Carr & Carr (1970), who examined cultured macrophages. The similarity of our SEM pictures of cryostat sections of epidermis in culture to those of authors who have examined other tissues in culture was both striking and surprising. The presence of a thin unicellular or subcellular membrane at the leading edge of the migrating column of cells emphasizes the similarity of epidermal cells in culture to other tissues *in vitro*.

Clearly, it is easy to be bemused by the visual appeal of SEM pictures and believe that this method of examination has merits merely because of its aesthetic quality. However, we believe that it gives information not obtainable with other methods; in particular concerning intercellular relationships and the detailed structure of membranes (albeit histologically fixed membranes). We submit that it would not have been possible to see the relationships of the dendritic cells with the neighbouring keratinocytes illustrated in the present communication in any other way. In the same way, it is doubtful whether the similarity in appearance of epidermal cells in organ culture to those of other tissues would have been seen using other methods. Apart from its diagnostic and research use in the examination of scale and hair we suggest that the SEM has considerable value for inspection of epidermal tissue, and that this capability has not as yet been generally recognized.

ACKNOWLEDGMENTS

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The association of pemphigus with thymoma or malignancy: a critical review

LAWRENCE S.KRAIN

Department of Dermatology, Harvard Medical School, Boston, U.S.A.

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SUMMARY

In order to determine the relationship between all forms of pemphigus and malignancy or thymoma, a search of the world literature was carried out. Fifty-nine case reports or isolated citations for pemphigus with malignancies or thymomas were noted, including twenty-one reports published before the histological separation by Lever of pemphigus and bullous pemphigoid in 1953. Of these cases, twenty-six were acceptable for analysis. In approximately 54% of patients surveyed with these associations, the malignancy involved the lymphoid or reticuloendothelial systems. Approximately 48% of the patients with pemphigus and malignancy or thymoma had associated autoimmune diseases. Pemphigus with malignancies or thymomas may be related through some intervening variables such as immuno-suppressive therapy or the antigenic and cross-reacting nature of the primary tumour, or through an immunological consequence of associated autoimmune disease.

Although reports of pemphigus and concurrent malignancy or thymoma have appeared sporadically in the literature (Skog, 1964; Beutner *et al.*, 1968; Krain *et al.*, 1973), added impetus to the study of these associations has been provided by the demonstration of the production of pemphigus antibodies by a lymphoid neoplasm (Saikia, 1972). It is generally acknowledged that autoimmune mechanisms play an important though as yet ill-defined role in the pathogenesis of pemphigus (Beutner *et al.*, 1968; Beutner, Chorzelski & Jordon, 1970), of thymomas (Stillman & Baer, 1972; Vetters *et al.*, 1973) and of some malignant tumours (Moragas, Winkelmann & Jordon, 1970; Alexander, 1972; Saikia, 1972). In addition, immunosuppressive therapy employed in the management of patients with pemphigus and other autoimmune diseases has been incriminated as a possible inductive mechanism in the subsequent development of malignancy (Harris, 1971; Lowney, 1972; Krain & Bierman, 1973). Saikia (1973) has suggested that every patient with pemphigus should be investigated as far as is practicable to exclude the possibility of a co-existent malignancy, especially in those cases in which systemic corticosteroid therapy is unsatisfactory in controlling the pemphigus.

The purpose of this paper is to review the current literature on the association of pemphigus with malignancy or thymoma and to examine critically the factors involved in these inter-relationships.

Address for correspondence and reprints: Dr L.S.Krain, Massachusetts General Hospital, Fruit Street, Boston, Massachusetts 02114, U.S.A.

TABLE I. Temporal association of various forms of pemphigus with lymphoid or malignant neoplasms

Neoplasm by organ site	Reference	Pemphigus developed before/after neoplasm	Time interval (months)	Type of pemphigus	Comments
1. Carcinoma of the ovary	Ryan (1971)	After	Unknown	P. vulgaris	Author stated no apparent relationship between the two entities
2. Carcinoma of the stomach	Lever (1965)	Before	Unknown	P. vulgaris	
3. Scirrhous carcinoma of breast	Krain & Bierman (1973)	After	1	P. vulgaris	
4. Scirrhous carcinoma of breast with regional node metastases	Krain & Bierman (1973)	Before	20	P. vulgaris	
5. Carcinoma of the endometrium	Krain *et al.* (1973)	Before	12	P. vulgaris	
6. Choriocarcinoma of the uterus	Elliott (1938)	Unknown	Unknown	P. vulgaris	
7. Metastatic squamous cell carcinoma of urinary bladder	Krain & Bierman (1973)	After	12	P. vulgaris	
8. Squamous cell carcinoma of oesophagus	Shecklakov (1966)	Unknown	Unknown	P. vulgaris	
9. Probable metastatic neoplasia in liver and squamous cell carcinoma of lip	Saikia & MacConnell (1972)	Unknown	Unknown	P. erythematosus	A liver scan showed findings consistent with widespread neoplastic involvement but cirrhosis could not be excluded

10. Malignant neoplasm of bronchus (type unknown)	Saikia & MacConnell (1972)	Before	15	P. erythematosus	No biopsy evidence of tumour but cytology revealed clumps of tumour cells
11. Adenocarcinoma of bronchus	Saikia & MacConnell (1972)	After	13	P. erythematosus	
12. Kaposi's sarcoma	Pisanty & Garfunkel (1970)	Before	20	P. vulgaris	
13. Kaposi's sarcoma	Rosenmann (1966)	Unknown	Unknown	P. vulgaris	
14. Lymphosarcoma	Krain & Bierman (1973)	Before	10	P. vegetans (vulgaris)	
15. Giant follicular lymphoma	Saikia (1972)	After	1	P. vulgaris	Lymphoid tumour produced excessive IgG in intercellular spaces of stratified squamous epithelium
16. Lymphoblastoma, sarcomatous type	Baird (1936)	After	12	P. vulgaris	Clinical but no biopsy evidence of pemphigus
17. Lymphosarcoma	Muller, Herrmann & Winkelmann (1972)	After	12	P. vulgaris	Pemphigus developed after cobalt therapy for lymphosarcoma
18. Epidermoid carcinoma in leg ulcer	Saikia (1973)	Before	Unknown	P. vulgaris	

MATERIALS AND METHODS

A Medlars (National Library of Medicine) search of the world literature since 1965 was carried out and supplemented by a manual search of the literature before 1965. Fifty-nine case reports or isolated citations for pemphigus with malignancies or thymomas were noted, including twenty-one reports before the histological separation by Lever (1953) of pemphigus and bullous pemphigoid. Of these cases, only the twenty-six noted in Tables 1 and 2 were acceptable for analysis. Thirty-three cases were excluded because of insufficient clinical and/or histological data for the diagnosis of either the pemphigus, the malignancy or the thymoma.

RESULTS

Tables 1 and 2 indicate those literature citations which were acceptable for analysis. Of the twenty-six acceptable associations, nineteen patients (73%) had a diagnosis of pemphigus vulgaris, while six (23%) patients had pemphigus erythematosus and one had pemphigus vegetans. The malignancies associated with pemphigus of all types included lymphomas (four), Kaposi's sarcomas (two), uterine malignancies (two), breast carcinomas (two) and bronchogenic carcinomas (two), along with a variety of other tumours. Table 2 lists those benign and malignant thymomas that were associated with pemphigus (eight cases). Table 3 shows that corticosteroid therapy preceded the malignancy in 35% of the twenty-six patients with pemphigus. Doses of corticosteroids varied from 30 to 120 mg daily of oral prednisone for periods varying from 2 to 108 months (35 months average).

DISCUSSION

Krain & Bierman (1973) have recently used the criteria of Curth (1971) to show that a causal relationship may exist between pemphigus and internal malignancy or thymoma.

Tables 1 and 2 show that pemphigus developed after the neoplasm in nine cases; in ten cases pemphigus developed before the neoplasm, and in seven cases there was insufficient information to ascertain which came first. Pemphigus followed the development of malignancy by a period varying from 1 to 13 months while it preceded the development of internal malignancy in those patients receiving corticosteroids (Table 3) by a period of 2 to 108 months. However, as pemphigus malignancy or thymoma may have long latent periods before they are diagnosed clinically, it may be difficult to determine accurately which disease arose first.

The frequent association of pemphigus with thymomas, particularly those of the spindle cell type (Stillman & Baer, 1972; Vetters *et al.*, 1973), shown in Table 2, suggests an immunological relationship. Five of the eight cases in this table of thymoma and pemphigus also had myasthenia gravis. Since thymic tumours have been reported in approximately 10–20% of patients with myasthenia gravis and 25% of such thymomas are clinically malignant (Bennett, 1966; Castleman, 1966; Stillman & Baer, 1972), the association in our review of the three conditions (i.e. thymoma, pemphigus and myasthenia gravis) suggests that pemphigus is more than coincidentally related to thymomas and myasthenia gravis. Stillman & Baer (1972) have previously postulated that the occurrence in post-natal life of thymomas so changes the thymic milieu that autoantigens which are present in the thymus (i.e. cell nuclei, myoid cells, and squamous epithelium) have an immunological rather than a tolerogenic effect. The result of this thymic aberration could be the formation of antibodies against cellular constituents. This theoretical construction might explain the association between thymomas and such autoimmune diseases as systemic lupus erythematosus, myasthenia gravis and bullous skin diseases. In Tables 1 and 2, five patients had myasthenia gravis and seven had lupus erythematosus, i.e. approxi-

TABLE 2. Temporal association of various forms of pemphigus with thymomas (benign and malignant)

Reference	Comments	Type of pemphigus	Pemphigus developed before/after neoplasm	Time interval (months)	Thymoma type	Myasthenia gravis
1. Kough & Barnes (1964)	45-year-old male with associated erythroid aplasia and positive L.E. cell phenomenon	P. vulgaris	After	27	Benign spindle cell type	Absent
2. Peck *et al.* (1968)	43-year-old female, intercellular fluorescence noted but may be false, positive finding as patient never developed pemphigus clinically	P. vulgaris	Unknown	Unknown	Malignant	Present
3. Beutner *et al.* (1968)	45-year-old male had thymectomy	P. erythematosus	After	1	Unknown	Present
4. Beutner, Chorzelski & Jordon (1970)	30-year-old female with myasthenia gravis for 18 years. No biopsy evidence of thymoma	P. erythematosus	Before	60	Unknown	Present
5. Jablonska, Chorzelski & Blaszyzyk (1970)	40-year-old male	P. erythematosus	Unknown	Unknown	Unknown	Present
6. Stillman & Baer (1972)	62-year-old male	P. vulgaris	Before	6	Benign	Absent
7. Krain & Bierman (1973)	52-year-old male	P. vulgaris	Unknown	Unknown	Unknown	Absent
8. Vetters *et al.* (1973)	46-year-old female	P. vulgaris	Before	9	Benign spindle cell type	Present

TABLE 3. Temporal and dosage relationship of corticosteroid therapy for pemphigus to the development of subsequent neoplasms

Neoplasm	Reference	Duration of corticosteroid therapy before neoplasm was diagnosed (months)	Corticosteroid dose range (daily)	Time interval between diagnosis of pemphigus and neoplasm	Comments
1. Calcified benign thymoma	Kough & Barnes (1964)	23	30–40 mg prednisone orally	27	P. vulgaris
2. Thymoma	Beutner, Chorzelski & Jordon (1970)	Unknown	Unknown	60	P. erythematosus
3. Thymoma	Vetters *et al.* (1973)	Unknown	Unknown	9	P. vulgaris
4. Epidermoid carcinoma in leg ulcer	Saikia (1973)	Unknown	20–40 mg prednisone orally	Unknown	P. vulgaris
5. Kaposi's sarcoma	Rosenmann (1966)	Unknown	Unknown	18	P. vulgaris
6. Kaposi's sarcoma	Pisanty & Garfunkel (1970)	2	Prednisone 30 mg orally	20	P. vulgaris
7. Lymphosarcoma	Krain & Bierman (1973)	108	Prednisone 7 5–80 mg orally	108	P. vegetans (vulgaris)
8. Scirrhous carcinoma of breast	Krain & Bierman (1973)	8	Prednisone 60 mg orally	20	P. vulgaris
9. Carcinoma of the endometrium	Krain & Bierman (1973)	12	Prednisone 120 mg orally to 10 mg 4 times daily	12	P. vulgaris

mately 46% of the patients had associated autoimmune diseases. Lymphoproliferative disorders have also been shown to appear with increased frequency in systemic lupus erythematosus (Hench, 1962). However, the statistical probabilities of the co-existence of pemphigus, systemic lupus erythematosus, and thymoma are so small that its not uncommon occurrence must be considered significant.

Of the total of twenty-six malignancies or thymomas associated with pemphigus, approximately fourteen or 54% appeared to involve the reticuloendothelial system. For the purposes of this discussion, Kaposi's sarcoma is considered a tumour of the reticuloendothelial system (Uys & Bennett, 1959). Thus, one possible explanation for the association of pemphigus with malignancy or thymoma, is that they are co-existing autoimmune phenomena.

Another possible explanation for the association of pemphigus with malignancy or thymoma is the suggestion of Saikia (1972) that the malignant tumour tissue produces excessive IgG type of antiepithelial antibodies and is responsible for the production of pemphigus antibodies present in the patient's serum. It is conceivable that the patient described by Saikia may have had subclinical pemphigus (Beutner, Chorzelski & Jordon, 1970) at some time before the development of the lymphoma.

Another theory for this association relates to the fact that some carcinomas have antigenic determinants similar to those found in the intercellular spaces of the epidermis, and that antigenic cross-reactions may occur. Moragas *et al.* (1970) have shown that the intercellular substance present in benign neoplasms, as well as in normal epidermis and in the skin of patients with pemphigus, binds pemphigus antibody and appears to correlate with the differentiation of the basal cell into the true keratinocyte. However, they also found that the intercellular substance was absent in squamous cell carcinoma and that this absence was related to the degree of neoplasia. However, as there are many tumour specific antigens (Alexander, 1972) and more than one type of antibody in various neoplasms, and since Moragas and his colleagues used only conjugated antiserum to human IgG, additional studies are needed to examine cross-reaction possibilities between pemphigus sera and various tumours. A recent case report from Formosa (Chang *et al.*, 1973) has demonstrated evidence of an association between epithelial malignancy and the production of antibodies to epithelial tissue.

A fourth possibility which might explain the relationship of pemphigus and malignancy is that corticosteroid therapy preceded the malignancy in 35% of the twenty-six patients (Table 3). Corticosteroids are known to enhance the development of various malignancies in animals (Anbari, Shklar & Cataldo, 1965; Shklar, 1966) and to promote metastasis (Albert & Zeidman, 1967). Systemic corticosteroids and other immunosuppressive agents, such as methotrexate, cyclophosphamide, and azathioprine, which represent the mainstay of long-term therapy of patients with pemphigus, also suppress the immunological surveillance mechanisms governing the host's defense against neoplasia (Harris, 1971; Lowney, 1972).

Corticosteroids have been alleged to predispose individuals to malignancy by several postulated mechanisms, including (a) a direct oncogenic effect (Shklar, 1966), (b) as a co-carcinogen potentiating environmental carcinogens (Anbari *et al.*, 1965), (c) by inducing suppression of humoral and cell-mediated immunity governing cancer surveillance (Burnet, 1970), and (d) by allowing, by means of a derepressor mechanism, latent oncogenic viruses to proliferate (Burnet, 1970; Fahey, 1971).

It is important to note that there are few reported associations of malignancies in patients receiving long-term corticosteroids for benign disorders such as rheumatoid arthritis (Skinner & Schwartz, 1972). However, it is notable that extremely high doses of corticosteroids are often employed in patients with pemphigus and the resulting profound lymphopenia, and depression of cell-mediated immunity and humoral antibody production may play a significant role in the subsequent development of malignancies. The oncogenic effect of high doses of corticosteroids in patients with pemphigus cannot be accurately evaluated, since as many as 37·5% of such patients succumb from side effects of steroid therapy, long before malignant neoplasms might otherwise have time to develop (Lever, 1965; Ryan, 1971). Recipients of renal allografts receiving immunosuppressive therapy with prednisone and azathioprine develop a statistically significant increase in frequency of neoplasms (fifty-two neoplasms including twenty-two lymphomas) out of 5000 recipients of allografts (Schneck & Penn, 1971). Harris (1971) has noted that chronic immunosuppression by methotrexate and/or corticosteroids may favour tumour emergence, growth and metastasis in man, and cites a communication from Starzl indicating that carcinomas occur as frequently as lymphomas or reticulum cell carcinomas in immunosuppressed patients.

Thus, although the exact pathophysiological mechanisms for the association of pemphigus and malignancies or thymomas are uncertain, inter-relations between autoimmune aberrations, immunological cross-reactivity and immunosuppressive therapy may be important determinants.

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Nikolsky sign on cervix uteri in pemphigus

F.SAGHER, B.BERCOVICI* AND R.ROMEM

Department of Dermatology and Venereology and *Department of Gynecology, Hadassah University Hospital, Jerusalem, Israel

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SUMMARY

Women suffering from pemphigus or from benign mucous membrane pemphigoid were examined gynaecologically. In thirteen of sixteen in whom the test was carried out, Nikolsky's was positive on the cervix uteri. It was consistently negative in control subjects.

Between 1954 and 1970, fifty-five women suffering from pemphigus or benign mucous membrane pemphigoid were hospitalized in the Department of Dermatology and Venereology of the Hadassah University Hospital, Jerusalem. They were routinely examined gynaecologically and sixteen were tested for Nikolsky's sign of the cervix uteri.

The Nikolsky sign on the skin is defined as the ready removal of part of the epidermis following a slight injury. When this test is applied to the normal-looking skin in pemphigus, the superficial part of the epidermis may be dislodged by a sliding movement of the finger. If this test is applied to a blister, the periphery of the blister can sometimes be enlarged, as has been shown by Asboe-Hansen (1960).

METHODS

In order to elicit the Nikolsky sign on the cervix uteri the following method was used.

The cervix uteri of pemphigus patients was exposed by the introduction of a self-retaining vaginal speculum. A gentle to moderate pressure was applied to the cervical surface with the aid of a cotton-tipped applicator. As a result of this pressure local hyperaemia or a slight erosion appeared. Only if an elevation of a portion of the mucosa occurred was this interpreted as a positive Nikolsky sign.

On another place on the cervical surface an attempt was made to peel off a portion of the epithelium with the aid of dissecting forceps. A denuded area following the elevation of a mucosal fold was interpreted as a positive Nikolsky sign.

FINDINGS

Of the sixteen cases tested for the Nikolsky sign of the cervix uteri, thirteen gave a positive sign and in three it was absent.

Of the negative cases, two had active skin and oral lesions of pemphigus vulgaris; the third was a patient in an inactive stage of the disease, who had been admitted to the department for control of steroid therapy. Of the thirteen patients presenting a positive Nikolsky sign of the cervix uteri, eleven had pemphigus vulgaris, one had pemphigus erythematosus of the Senear-Usher type, and one suffered from benign mucous membrane pemphigoid.

Age

The ages of the patients ranged from 22 to 59 years; two thirds were between 22 and 44 years.

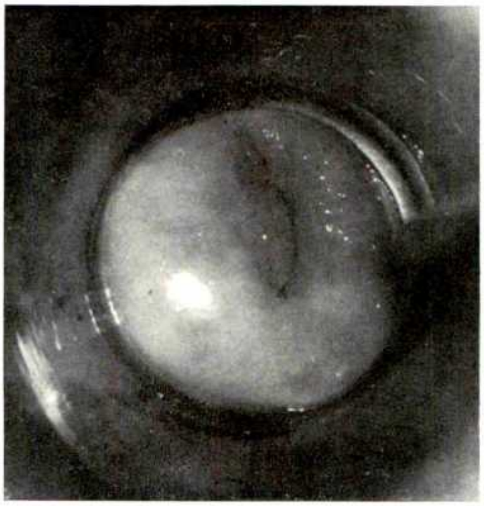

FIGURE 1. Pemphigus vulgaris; cervix uteri before Nikolsky sign was induced.

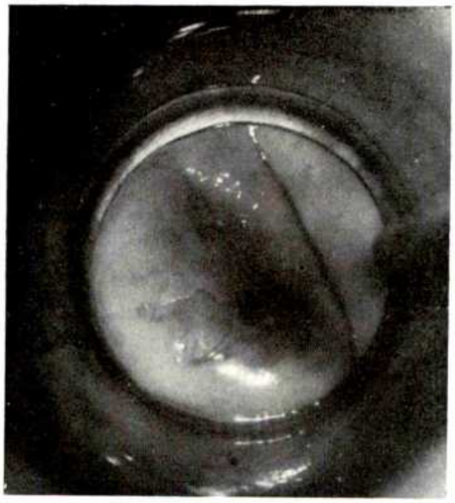

FIGURE 2. Same patient; a positive Nikolsky sign was elicited by lifting up part of the mucosa on the left lower part of the cervix uteri.

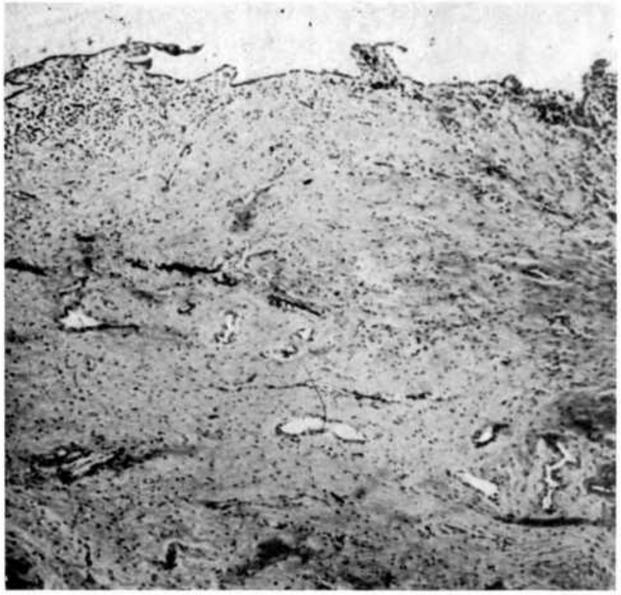

FIGURE 3. Histological examination of the site of a positive Nikolsky sign on the cervix uteri reveals the partial loss of the mucosa.

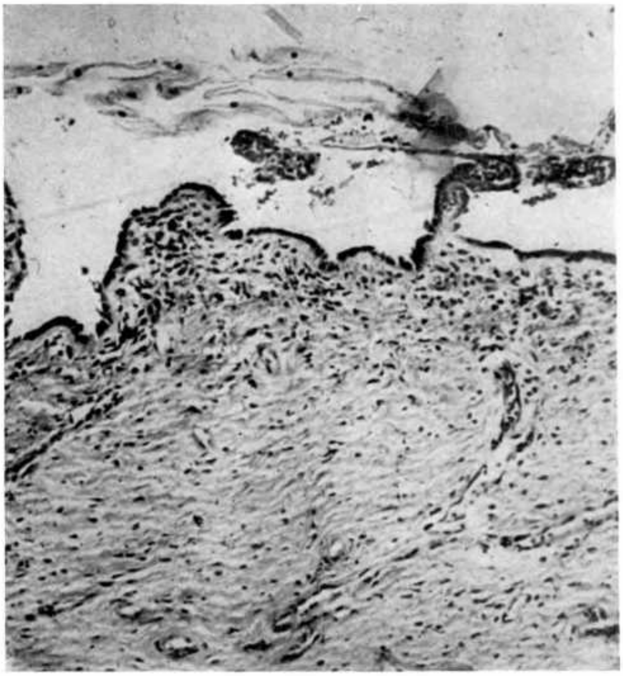

FIGURE 4. Same case as in Fig. 3. Higher magnification. Finger-like protrusions of the papillae are present; the basal cell layer of the mucosa remains and covers the whole surface.

CONTROLS

In order to evaluate the results obtained in pemphigus patients, the same methods of examination were applied to 102 gynaecological outpatients, attending with routine gynaecological disorders, functional or organic, for investigation or for routine check-up.

Two of the controls were younger than 20 years of age; twenty-nine were in the 21–30 age group, forty-four in the 31–40 age groups, sixteen in the 41–50 age group and eleven were over 50 years of age.

By using the applicator method, in fifteen patients a local hyperaemia and in six a slight erosion was induced. By the forceps method, in sixteen a small erosion was produced; six of these patients were over 50. In none of the control patients was a clear-cut positive Nikolsky sign obtained.

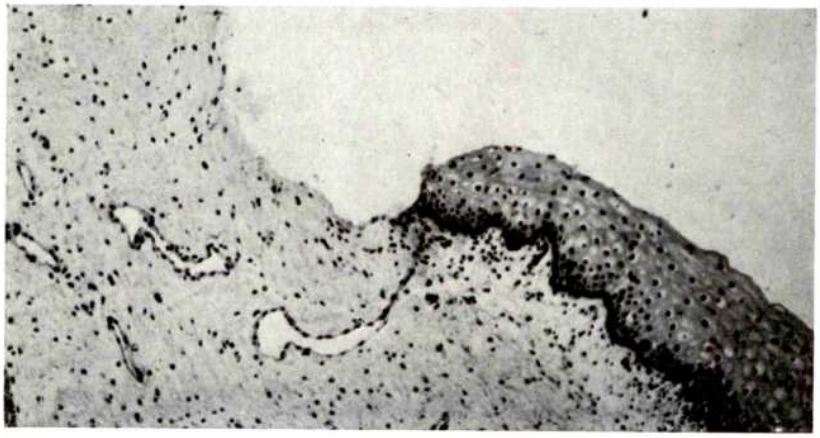

FIGURE 5. Control case; traumatic erosion revealing a complete loss of the epithelial lining without acantholysis.

Histological examinations

Biopsies were taken from the site of the positive Nikolsky sign on the cervix uteri in six cases of pemphigus. In two control cases in which an erosion was produced by pressure, biopsies were also taken from these sites.

The histological examination revealed changes corresponding to those seen in the histology of the Nikolsky sign in the skin. The whole mucosal layer above the basal cell layer was removed and the papillae were finger-like protrusions covered by the basal cell layer.

DISCUSSION

A positive Nikolsky sign may be found in the skin in all types of pemphigus at certain stages. It occurs also in a number of dermatological disorders other than pemphigus, such as benign familial chronic pemphigus of Hailey–Hailey, benign mucous membrane pemphigoid, and epidermal necrolysis, and sometimes in dermatitis herpetiformis and epidermolysis bullosa.

The first observation of pemphigus lesions on the cervix uteri and a positive Nikolsky sign in this area was presented at the International Congress of Dermatology in Stockholm in 1957 (Sagher, 1957). A paper was read before the Pacific Dermatologic Association in 1965 on the observation in five cases (Sagher, 1965, 1966). Mikhail gave a clinical description of a case of pemphigus vulgaris involving the cervix uteri in 1967.

In the present series of patients with pemphigus, including one case of benign mucous membrane pemphigoid, a positive Nikolsky sign could be elicited in thirteen out of sixteen. This suggests that the cervix uteri is frequently involved clinically or subclinically during the active stage of pemphigus.

The test for the Nikolsky sign on the cervix uteri may be a sensitive indicator of activity of the disease. It should be noted that the variation in the number of active lesions and in the intensity of the disease process was considerable, some cases being in a very florid state and others having minimal lesions. Only one case of inactive pemphigus vulgaris was included in this series and in this patient the Nikolsky sign was negative on the cervix uteri as well as on the skin. In one patient with Senear-Usher type of pemphigus in an inactive state the Nikolsky sign on the cervix uteri was positive.

Further experience may indicate whether a positive Nikolsky sign of the cervix uteri precedes reactivation of the disease or is merely a concomitant manifestation of clinical activity.

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Basal cell hamartoma and cellular naevus: an unusual combined malformation

F.SCHELLANDER, R.MARKS AND E.WILSON JONES

The Department of Histopathology, The Institute of Dermatology, Lisle Street, Leicester Square,
London WC2H 7BJ

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SUMMARY

In this report are discussed three similar complex lesions which were composed of intermingled elements of a hamartomatous basal cell tumour and of a dermal cellular naevus. It appeared that some of the naevus cells arose directly from the basal cell strands in the deep portions of the hamartoma. This situation is contrasted with the coincidental origin of an aggressive basal cell epithelioma at the site of a dermal naevus. An instance of a dermal cellular naevus and an immediately adjacent syringoma is also described.

There have been few reports of cellular naevi occurring in combination with other tumours of epidermal or adnexal origin. Sigal & Saunders (1967) described two lesions, which they considered to be invasive basal cell epitheliomas, arising in and permeating cellular naevi. Notably, the lesions had not ulcerated and there had been no history of recent growth.

We do not think that Halkin's (1932) cases are similar to the group presented here. His cases were probably heavily pigmented basal cell epitheliomas and we are unconvinced that a separate naevus cell component had been demonstrated.

In this study we describe a number of lesions in which a basaloid or adnexal tumour and a cellular naevus occurred together. Three of the lesions were of particular theoretical interest because the two separate components of the tumours, strands of basaloid hamartoma and groups of naevus cells, roughly equal in proportion, were so closely intermingled as to suggest a 'symbiotic' relationship. This situation is contrasted with the occurrence of an aggressive basal cell epithelioma arising at the site of a dermal cellular naevus, and with a syringoma and a dermal cellular naevus present in close proximity.

MATERIAL AND METHODS

Five cases were traced from the histological records and files of St John's Hospital. 5 μm thick sections were cut from paraffin-embedded material and stained with haematoxylin and eosin, toluidine blue, van Gieson's stains for collagen, orcein for elastic fibres and a reticulin stain.

Case 1

A white female, aged 31, had a firm skin-coloured nodule below the left lower eye-lid since the age of 16. There had been a recent increase in size but there was no evidence of ulceration. The lesion was excised *in toto*. No recurrence was reported.

Case 2

A 49-year-old white male had a slightly raised and pigmented lesion on the left cheek, which had been present for about 30 years. There was no history of recent growth or of ulceration. The lesion was excised *in toto*. No recurrence was reported.

Case 3

A 29-year-old white man had a small skin-coloured lesion on his left cheek which had a pigmented centre. Its duration was unknown. There was no history of recent growth and no ulceration. The lesion was excised *in toto*. No recurrence was reported.

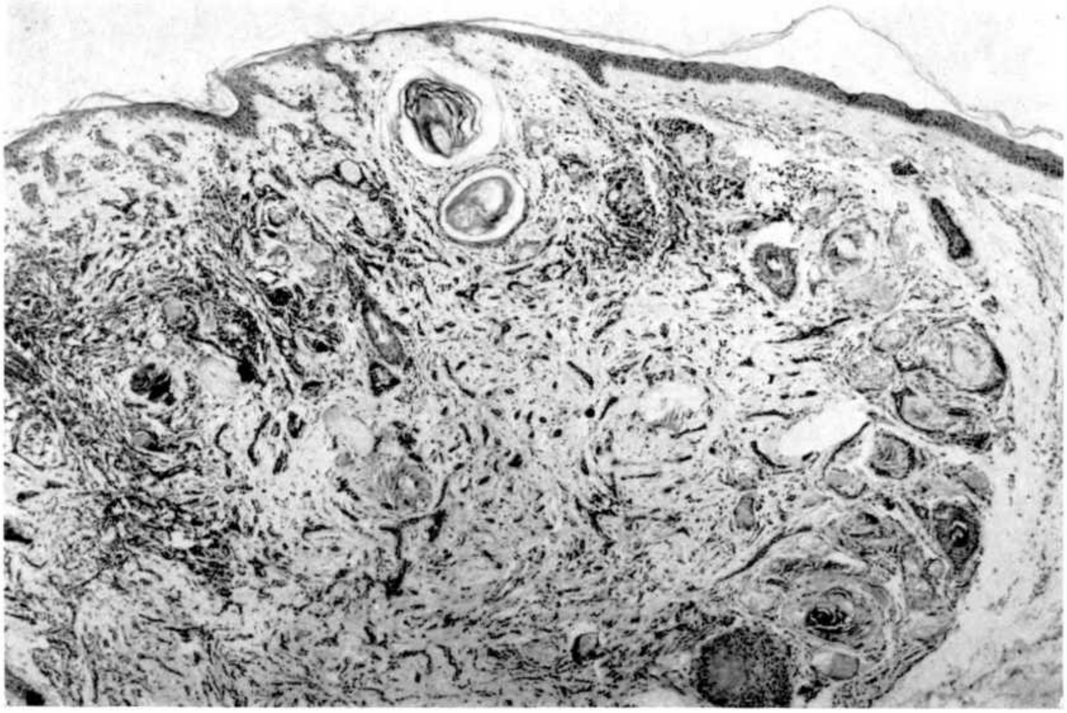

FIGURE 1. Low power view of lesion of case 1 to show the symmetry and sharp border of the tumour. There are numerous cystic spaces either filled with lamellar keratin, shadow cells or calcified contents. Naevus cell nests merge imperceptibly with basaloid tumour strands (H & E, × 20).

Histopathology

The histological features of cases 1–3 were so similar that they can be described together. Each lesion consisted of a roughly symmetrical and well demarcated tumour, which occupied the entire depth of the dermis (Figs. 1, 2 and 3). They were composed of two distinct components or 'cell lines': strands and columns of basaloid cells, and dispersed nests of dermal naevus cells. At the upper border, some of the basaloid strands were in direct continuity with the epidermis. These strands were usually slender, being only 2–3 cell rows wide. In places branching was observed, and a number of small keratin filled cystic dilatations were noted, somewhat reminiscent of trichoepithelioma. In places the contents of the cysts had undergone 'mummification' with loss of nuclear staining, leaving 'shadow cell' remnants. Some of the cysts had undergone calcification, particularly towards the periphery of the lesions. In general the naevus cells were present either in compact faceted cell nests as in ordinary dermal naevi, or in a more loosely dispersed arrangement. There was some tendency for the cells of the dispersed element to be fusiform in shape and for the cells to be orientated in columns or files in very close proximity to the basaloid strands. Many of the strands of basal cells showed individual 'clear' cells and undoubted evidence of 'junctional' activity, with small groups of naevus cells budding off from the sides of the epithelial columns (Fig. 4). In places the two separate cell components were so closely intermingled that it was difficult to tell to which component some of the individual cell groups belonged. In two of the lesions no junctional activity was noted from the epidermis but epidermal clear cells were prominent. In the remaining lesion (case 2), several theques of pigmented naevus cells were present at the dermo-epidermal junction over the nodule. In this particular lesion there was at one margin an area of pure dermal cellular naevus; in the rest of the tumour the proportion of naevus cells to the basaloid component varied considerably from place to place.

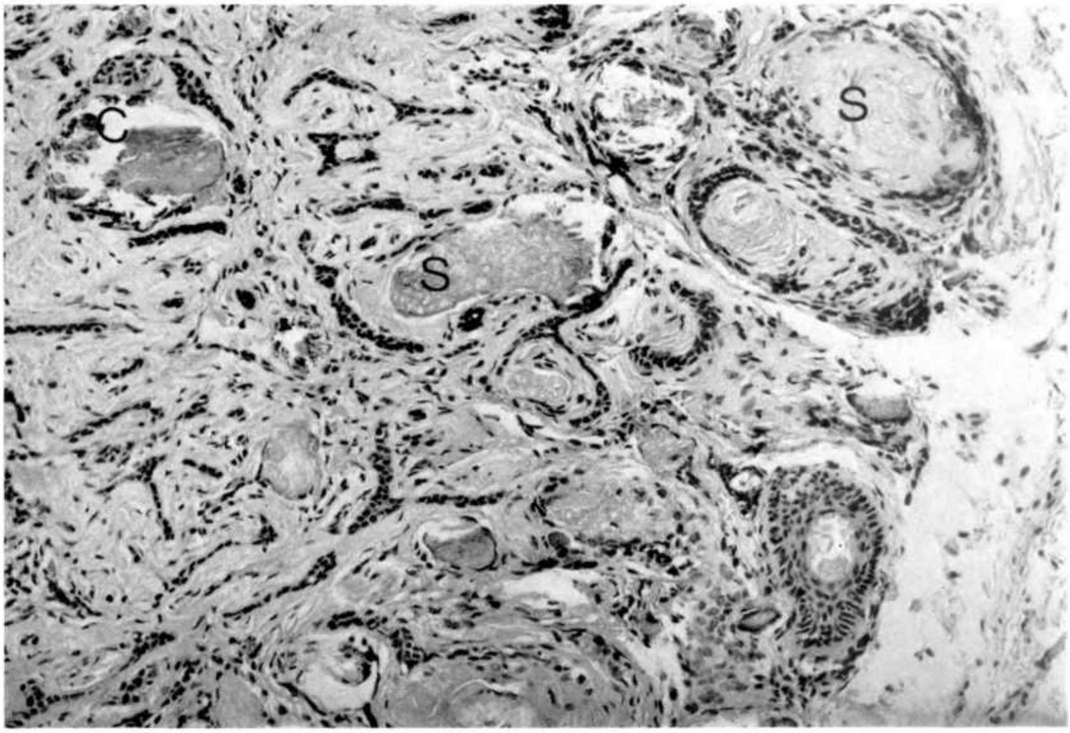

FIGURE 2. Detail of Fig. 1 showing a calcified cyst (C), several spaces filled with 'shadow cells' (S) and delicately branched strands of basaloid cells (H & E, × 32).

There were few elastic fibres within the lesions. The collagenous matrix of the tumours surrounded the basaloid strands in hyalinized sheaths. The PAS stain emphasized the well-marked hyaline stroma which sleeved the basaloid component. Metachromatic stains did not reveal an excess of mast cells. There was neither an associated inflammatory cell infiltrate nor scarring, and small vessels were not prominent within the nodules. Silver stains showed that melanin was confined to the epidermis and a few superficial macrophages in two of the lesions. In case 2 several of the superficial theques of naevus were melanized but no melanin was found at deeper levels.

Case 4

A 46-year-old white man had a small skin coloured nodule, which had been present for an unknown period, on the bridge of the nose. It was excised for cosmetic reasons. No recurrence was reported.

Histopathology

The lesion consisted of a simple dermal cellular naevus, which was partially replaced by fatty tissue, and, immediately adjacent but not intermingled, a typical syringoma. The overlying epidermis was normal.

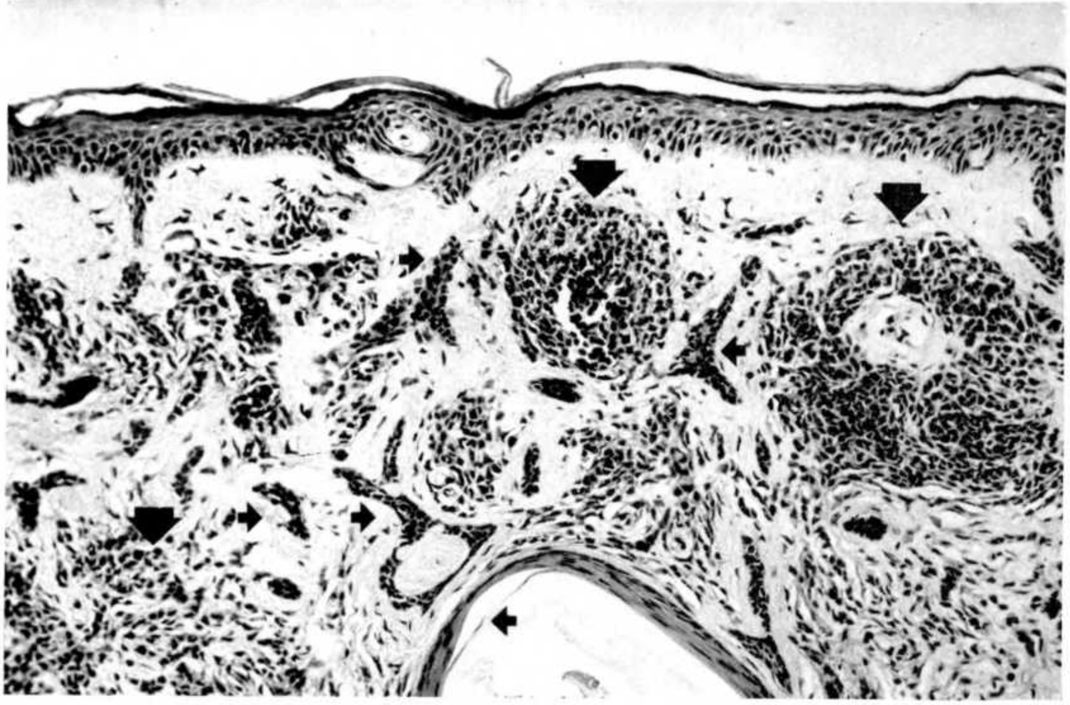

FIGURE 3. Detail of case 2 to show the relationship between the naevus cell nests (large arrows) and basaloid strands (small arrows) in upper part of the lesion. There is also a large cyst of the type found in trichoepitheliomas (H & E, × 32).

Case 5

A 55-year-old white woman had a small flat lesion, with a pearly raised edge and a recurrent scab, of only 2 months' duration. Clinical diagnosis: basal cell epithelioma.

Histopathology

A typical basal cell epithelioma had extended to the lower dermis. Towards its centre it was ulcerated. It consisted of typical interlacing strands and lobules of basal cells showing peripheral palisading.

Some tendency to horn pearl formation was present. Towards the periphery and lower dermis, the features were those of a morphoeic basal cell epithelioma with narrow strands of infiltrating cells separated by a loose and moderately cellular connective tissue stroma. The overall appearance was that of an aggressive tumour. Towards one side of the epithelioma there was a small dermal cellular naevus below the epidermis. Strands of basal cell epithelioma had infiltrated between the naevus cell nests and in places were enclosing and surrounding small groups of these cells (Fig. 5). The ulceration had extended to the margin of the naevus and the impression was that a pre-existing naevus was being destroyed by the invading epithelioma.

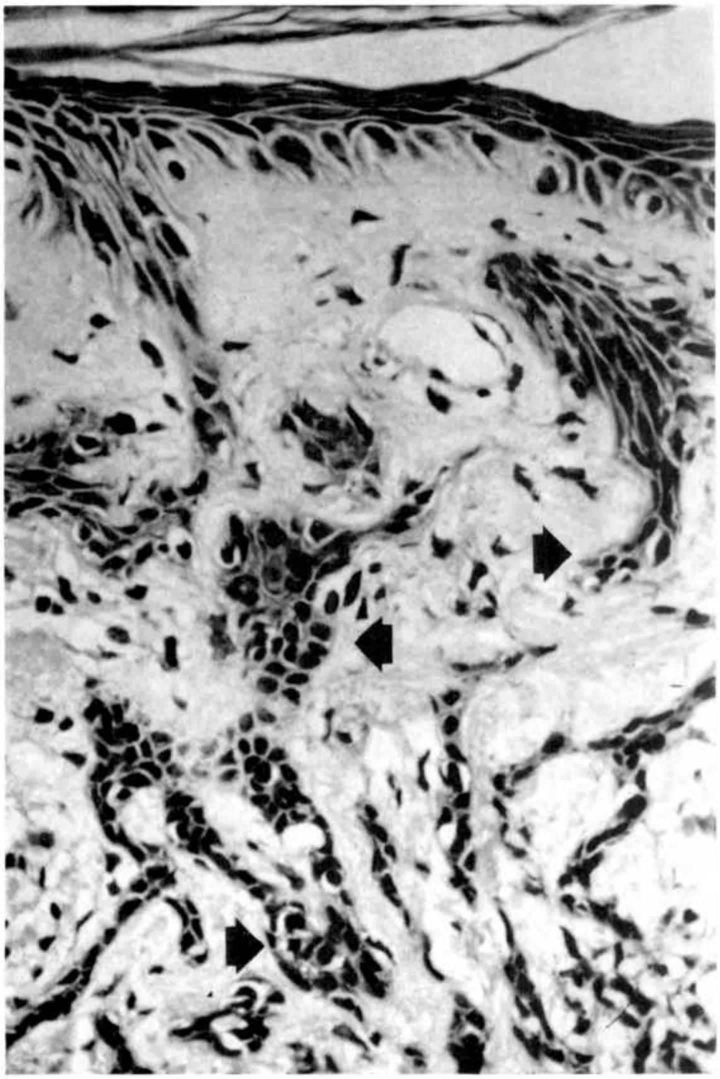

FIGURE 4. Detail from case 3 showing the intricate relationship between the delicate strands of basaloid tumour cells of epidermal origin and the naevus cells accompanying them in a 'junctional' pattern (arrows) (H & E, × 120).

DISCUSSION

We believe our first three cases are unusual in that two quite distinct tissue elements, naevus cells of neuroectodermal derivation and a basal cell hamartoma of ectodermal orgin, were contained in a single lesion. Our fifth case, in contrast, appeared to be an ordinary basal cell epithelioma arising adjacent to a cellular naevus and subsequently invading it. It is notable that this patient was the oldest in the group and that the clinical appearance and the history had suggested an invasive and destructive lesion. We do not believe that the pathology of our first three cases can be interpreted in a similar way; in these cases it seemed that the naevus cell element was secondary and was taking its origin from the basal cell component.

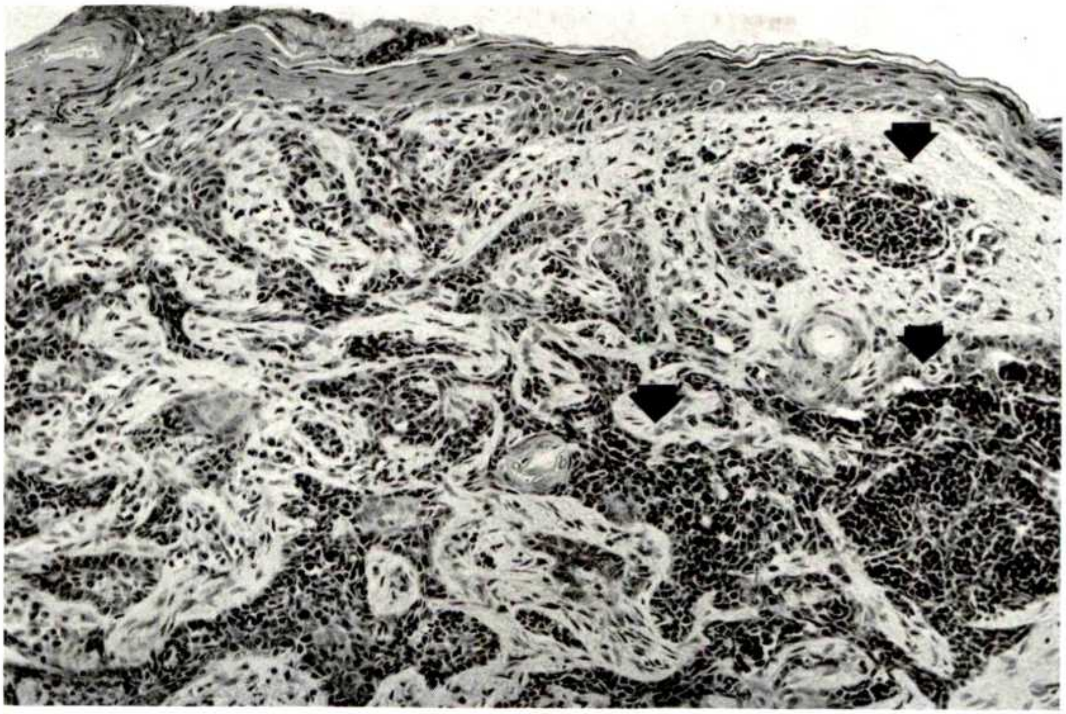

FIGURE 5. Case 5. This is an ulcerated invasive basal cell epithelioma with irregular tumour strands and keratinizing tendency. On the right half of the photograph naevus cell nests (arrows) are seen being invaded by the tumour (H & E, × 32).

In support of our view that the basal cell element in these tumours is hamartomatous rather than aggressive were the length of the clinical history and the absence of ulceration. Furthermore, the lesions as a whole were symmetrical and well demarcated and there was no inflammation or mucocellular stromal reaction, as is usual in ordinary basal cell epitheliomata. In addition, we had the impression that the individual cells were more evenly arranged than in an epithelioma of the morphoeic type, and palisading was not a feature. There was some evidence of differentiation towards adnexal structures similar to that seen in trichoepithelioma. Mummification and calcification in some of the tumour lobules also speaks for a tumour of a very indolent character.

It is surprising, considering the frequency of naevoid tumours and malformations of the skin and the wide range of possibilities they exhibit, that those derived from different embryonic germ layers are rarely associated. For instance, Wilson Jones & Heyl (1970) in their comprehensive study of naevus sebaceus and similar complex organoid malformations of the skin found no examples in which there was also a cellular naevus, and they could find only one well documented example of such an association in the literature, the case of Wechsler & Fisher (1965).

Case 4 presents a close association of a dermal cellular naevus and a syringoma, in which the two lesions abutted on each other, but with no actual intermingling of the two elements as in our first three cases. This could be a chance association, but it is to be noted that the patient had no other syringomas.

At present there is little detailed knowledge of the frequency and distribution of melanocytes in epithelial tumours of the skin. It is perhaps generally assumed that most of these tumours are unable to harbour melanocytes either because, in malignancy, the tissues are too disorganized or in hamartomas because of a differentiation towards adnexal structures, which in their mature state do not contain these cells. It is to be remembered that, in adult skin, melanocytes are limited to the basal cell layer of the epidermis, the wall of the follicular ostia above the level of the sebaceous gland ducts and the hair bulbs.

It is in keeping with this distribution that a few pilomatrixomas contain melanocytes as do some basal cell epitheliomas in contrast they are absent in most adnexal tumours. The occurrence of pigmented nodular hidradenomas is rare but has been observed (Wilson Jones, 1971), indicating that, exceptionally, some sweat gland hamartomas can tolerate an accompanying melanocyte population. It is of interest that Follman (1969) has described deep seated naevi in which he believed the naevus cells had arisen directly from the coiled part of the sweat glands themselves, implying that the glands must have had a preceding ectopic melanocyte population.

At present we do not know how the symbiotic relationship between the melanocytes and keratinocytes is regulated or what factors are responsible for the disruption of this relationship in skin tumours. In the three tumours that we describe here, it is not only remarkable that a hamartoma sharing adnexal differentiation in the direction of trichoepitheliomas should carry melanocytes, but that melanocytes should then subsequently proliferate to give rise to a complex but integrated malformation composed of two embryologically distinct cell lines.

ACKNOWLEDGMENTS

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Histology simulating reticulosis in persistent nodular scabies

JOHN THOMSON,* THOMAS COCHRANE,† REBECCA COCHRAN AND ALEXANDER McQUEEN

*Department of Dermatology, The Royal Infirmary, Glasgow, †Heathfield General Hospital, Ayrshire, and Department of Dermatology, University of Glasgow, Glasgow

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SUMMARY

Six patients with persistent nodular scabies are described. Initially the diagnosis was in doubt, and thus a lesion was biopsied in each case. The histology bore a striking resemblance to that of malignant lymphoid neoplasm in five cases. In the absence of a clinical diagnosis it was reported as such by experienced skin histopathologists. In the remaining case an acarus was seen histologically, but the type and extent of the dermal infiltrate suggested a concomitant adverse drug reaction.

We feel that these histological appearances may be misleading and that even with accurate clinical details the true diagnosis may be made only on the subsequent progress of the patient.

Scabies is a common disease. The incidence of the condition in the dermatology out-patient clinics of Glasgow and South West Scotland is currently about 5%.

Normally the clinical history and examination of the patient are sufficient for the experienced dermatologist to suspect the diagnosis. The finding of the acarus by microscopic examination of scrapings from a burrow confirms the diagnosis, so that biopsies are rarely performed in routine cases of scabies.

However, some patients with scabies present in an atypical fashion. One such fashion is as persistent nodular scabies.

Five cases of persistent papules after scabies were described by Ayres & Anderson (1932). The individual lesions were pea-sized, red or brownish-red, infiltrated nodules, some with a central depression. Pruritus was a common feature. All five cases had lesions of the trunk, buttocks and axillae. All the males had scrotal lesions and both adult males had penile lesions.

Samman (1963) reported to the Royal Society of Medicine a 36-year-old male with a pruritic nodular eruption in the groins, lower abdomen and axillae, each nodule being surmounted by a burrow containing an acarus.

Berge & Krook (1967) described the clinical and histological features of five cases of persistent nodules following scabies. Four of these patients suffered typical intense pruritus, but one complained only of occasional itching. The lesions were brownish-red or bluish-red nodules, up to 12 mm in diameter. They were not present on the light exposed areas and were most numerous on the buttocks, peri-umbilical region, penis and in the axillae.

The histology of six cases of persistent nodular scabies is described.

CASE REPORTS

Case 1

S.K., a 65-year-old female, presented with very severe scabies. Microscopical examination of scrapings from a lesion revealed numerous acari. There were eczematous lesions on the trunk and limbs. The sister with whom she lived was also suffering from scabies at this time.

The patient was admitted to hospital, treated with 1% gamma-benzene hexachloride cream for three nights, and given oral diphenhydramine. Thereafter she was given 2½% hydrocortisone ointment followed by 10% sulphur ointment for three nights.

She was seen again 3 months later by which time she had developed peculiar granulomatous lesions scattered over her arms, around her neck and on her body. Biopsy of a lesion from the wrist was reported as follows: histological examination shows a dense dermal infiltrate, pleomorphic in nature, composed of eosinophils, histiocytes, plasma cells and aberrant reticulum cells. In the absence of pseudo-epitheliomatous hyperplasia, these features are more suggestive of a lymphoma such as Hodgkin's disease than a of reactive process.

Detailed haematological and biochemical investigations revealed no abnormality.

She returned 8 months later with a recurrence of eczema on her ankles and arms. Microscopical examination of scrapings of lesions on this occasion failed to demonstrate acari. Treatment topically with hydrocortisone and chlortetracycline ointments produced a rapid improvement. No further lesions have since appeared and she has remained well for 8 months.

Case 2

J.M., a 50-year-old male, was first seen at a general medical clinic complaining of pruritus on all areas below the neck, but especially in the groins and on the foreskin. The symptoms had been present for 2 months. His wife and son also had pruritus. A diagnosis of scabies was made on clinical grounds and the entire family was treated topically with 1% gamma-benzene hexachloride cream and 10% crotamiton ointment.

Two months later the patient returned to the dermatology clinic when he still had evidence of scabies and this was confirmed by microscopic demonstration of the acarus from a burrow. He was again treated with gamma-benzene hexachloride cream and 10% crotamiton ointment. 1 month later he was noted to be almost clear and was given 0·1% each of fluocortolone pivalate and hexanoate in a cream base (Ultradil) to apply.

Three months later he returned with a granulomatous eruption involving the chest, axillary folds, the perianal region and buttocks. A clinical diagnosis of post-scabietic granuloma was made. At that time full haematological and biochemical investigations revealed no abnormality. A chest X-ray was normal. Biopsy from a lesion on the abdominal skin was reported as follows: histological examination shows some acanthosis of the epidermis with areas of superficial necrosis. A rather pleomorphic perivascular infiltrate is seen, most noticeable around the sweat glands. It consists of lymphocytes, eosinophils, histiocytes with occasional plasma cells and reticulum cells. While these appearances may be derived from an allergic reaction to the acarus, lymphoid neoplasia cannot be confidently excluded on histological grounds. A repeat biopsy in 3 to 6 months' time is advised.

He was admitted to hospital and treated with 10% sulphur ointment followed by 10% crotamiton ointment. 3 weeks later his skin lesions had improved considerably.

During the next 8 months he was treated intensively with topical 0·025% fluocinolone acetonide and 1% hydrocortisone ointments. At one stage he was given methylprednisolone acetate by intramuscular injection.

The lesions gradually subsided and when the patient was reviewed 10 months later he was symptom free and normal on clinical examination.

Case 3

H.Y., a 55-year-old male, presented with a 2-month history of an itchy papular eruption, initially round the shoulders but spreading to involve axillae and groins. His daughter also had noted a similar itchy eruption and they both had received inadequate topical benzyl benzoate therapy. The patient had a maculo-papular eruption on the shoulders, axillae and groins, obviously excoriated. A few vesicles were present in the axillae. Biopsy from a lesion on the shoulder was reported as follows: an acute on subacute dermatitic reaction is seen with superficial micro-vesicle formation. Foci of acanthosis with elongation of the rete ridges overlie a dermis which contains a dense infiltrate permeating to the deepest part. It is pleomorphic with plasma cells, eosinophils, lymphocytes, histiocytes and reticulum cells present in large numbers. Large cell forms are seen and nuclear hyperchromatism with occasional mitotic activity can be detected (Fig. 1). These appearances are interpreted as those of a malignant lymphoid neoplasm.

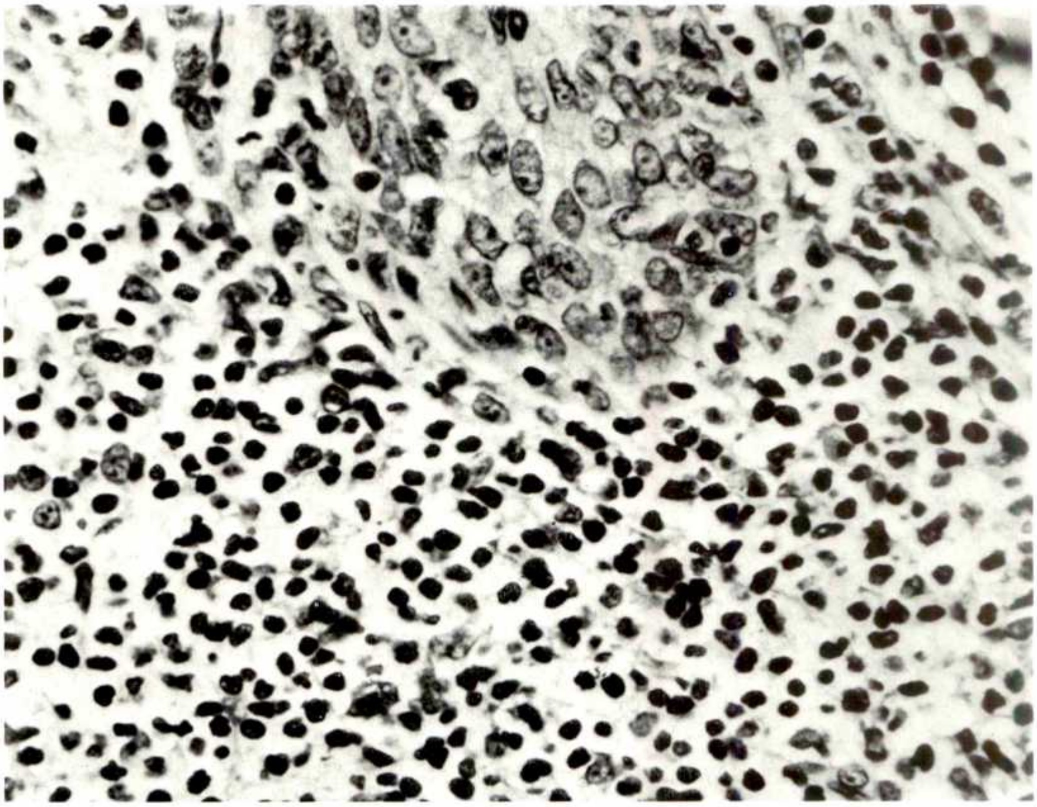

FIGURE 1. Case 3: shows pleomorphic dermal infiltrate with some large cell forms, nuclear hyperchromatism and occasional mitotic figures (H & E, × 400).

Over the next few months the patient developed no fresh lesions, but 4 months later the presence of lightly pigmented cutaneous nodules around the shoulders was noted. Over the following 3 months, fresh itchy lesions developed involving the trunk, hands, wrists and genitalia.

At this time his wife and son were treated by their general practitioner for an itchy skin eruption.

No details were available. He was admitted to hospital for investigation. On examination there were scabietic burrows on the penis, foreskin and finger webs. In addition firm reddish cutaneous nodules were present around the shoulders, buttocks, peri-umbilical region and scrotum (Fig. 2).

Full haematological and biochemical investigations revealed no abnormality. X-ray of the chest was within normal limits. The diagnosis of scabies was made and therapy with topical benzyl benzoate commenced. The patient responded symptomatically within 1 week. When reviewed 6 weeks later he had remained well.

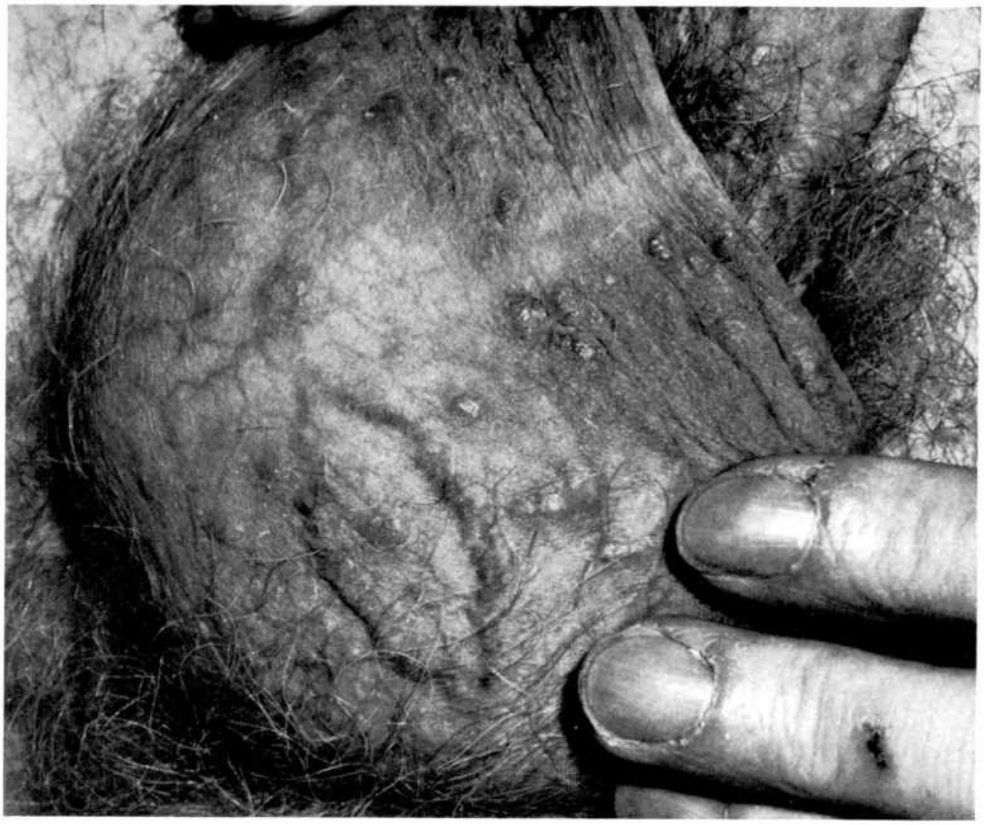

FIGURE 2. Case 3: shows nodular eruption on scrotum.

Case 4

M.R., a 60-year-old male, presented with an itchy rash of 3 weeks' duration which first appeared on his right thumb and spread to involve the arms, trunk and legs. The diagnosis at this stage was nummular eczema. He was given a weak tar preparation to apply; the rash improved, but he developed a few firm reddish nodules in the axillae. Later he became worse and the possibility of scabies was considered. He lived alone and thus there was no family history. An attempt to demonstrate acari microscopically in scrapings from the lesions was unsuccessful. He was nevertheless given anti-scabietic therapy in the form of topical 10% sulphur ointment. This had no effect on his condition, which remained static. A biopsy specimen 2 months after the onset of the condition was reported as follows: multiple sections of the material submitted showed widespread nodular aggregates of lymphocytes and histiocytes in the dermis with many eosinophils interspersed. The cellular infiltrate extends very deeply and in areas has reached the subcutaneous fat. Several germinal follicles are present. While these appearances may be derived from an insect bite, a diagnosis of Hodgkin's disease cannot confidently be excluded.

In view of the report he was admitted to the wards for further investigation. On examination he had widespread reddish nodules measuring up to 0·5 cm in diameter. These were indurated and intensely itchy. Minimal lymphadenopathy was present in the groins and left axilla but no other abnormalities were found on physical examination. Another nodule was biopsied. It was reported as follows: sections from the skin submitted show a dense infiltrate mainly confined to the upper dermis but which spreads down the vascular bundles to the subcutaneous fat. This infiltrate is pleomorphic in character and is composed of reticulum cells, histiocytes, lymphocytes, eosinophils and an occasional plasma cell. The appearances are those of a malignant lymphoid neoplasm, probably of Hodgkin's type.

Biochemical and haematological investigations, including a bone-marrow examination, revealed no abnormalities. A chest X-ray was within normal limits. Thus the diagnosis of a systematized lymphoma could not be substantiated.

Over the following 12 months the nodules regressed and no fresh lesions developed. During this time he was treated with topical 10% crotamiton ointment. He has remained symptom free for over 1 year.

The intense pruritus, the distribution of the eruption, the appearance of the individual lesions and the subsequent resolution were the features on which we diagnosed persistent nodular scabies. The sinister histology, similar to that of our other cases, with gradual resolution, would tend to corroborate this.

Case 5

G. McI., a 31-year-old male, was referred to the dermatology out-patient clinic with nodules on his scrotum. He was found also to have indurated, rather linear lesions on the flexor aspects of his elbows. No burrows were found.

At this time all other members of his family had pruritus and thus treatment for the entire group was instituted with 25% benzyl benzoate emulsion in the conventional manner.

When reviewed 3 weeks later, he still complained of itching. He was found to have scattered papular lesions all over the body, but no burrows. He was given one further application of 25% benzyl benzoate emulsion and thereafter 10% crotamiton and 0·25% hydrocortisone in a cream base.

Over the next 10 months he attended the out-patient department at intervals of approximately 1 month. He had papular indurated lesions on his buttocks, thighs, antecubital fossae and scrotum. These were most marked on the scrotum.

During this time his wife had two episodes of itching and his son one. At no time were any lesions found.

The patient was treated with topical 0·1% betamethasone 17-valerate ointment and oral trimeprazine tartrate. Subsequently he was given 10% sulphur ointment for topical application for 3 days.

The lesions persisted and a biopsy specimen was obtained from a lesion on his antecubital fossa. This was reported as follows: this shows a mild chronic dermatitic reaction with foci of subacute activity. Within the dermis there is a scattered, mainly perivascular cellular infiltrate which includes histiocytes, lymphocytes, and a few plasma cells, eosinophils and reticulum cells. Occasionally this infiltrate is seen to be aggregated around eccrine sweat glands and to extend to the depths of the section examined. No mitotic activity is seen but variability in cell size is noticeable and nuclei are hyperchromatic. No abnormal birefringence is seen on examination by polarized light. In general these histological appearances suggest that the patient should be screened for any possible lymphoid neoplasm in the absence of any firm history which might suggest an adverse drug reaction. Repeat biopsy in 3 months' time is felt to be necessary should clinical doubt persist.

For the next 6 months he was treated with oral trimeprazine tartrate and topical 10% crotamiton cream. The lesions and the symptoms subsided and the patient remained well.

Case 6

J.B., a 60-year-old male, presented with pruritus of 6 weeks' duration. On examination he had a papular eruption on the trunk and limbs. The papules were erythematous and excoriated.

Dermographism could be demonstrated. There was nothing of relevance in the family history and apart from ischaemic heart disease the patient's general health was good. Treatment prior to attendance at hospital had been topically 10% crotamiton, 0·25% hydrocortisone in a cream base, and systemic antihistamines. Biopsy of a lesion from the hip was reported as follows: histological examination of the skin submitted reveals an acarus within the stratum corneum. There is an acute urticarial reaction in the dermis associated with some free haemorrhage. It is not possible on histological grounds to assess whether this is related to the acarus or whether there is an associated drug eruption (Fig. 3).

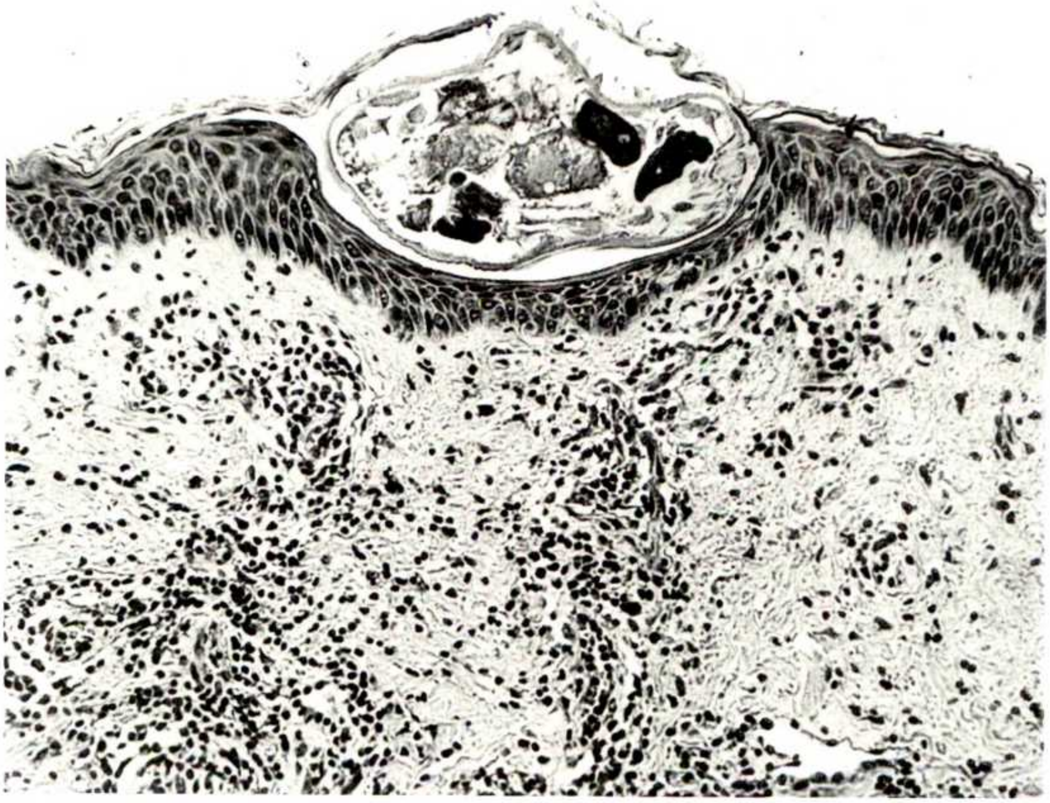

FIGURE 3. Case 6: shows an acarus in the keratin layer and pleomorphic dermal infiltrate (H & E, × 64).

Full haematological and biochemical investigations revealed no abnormality. X-ray of the chest was normal. The patient was treated with topical benzyl benzoate and when reviewed 3 weeks later was free of itch and there were no cutaneous lesions present.

DISCUSSION

The diagnosis of scabies was made on clinical grounds. It two cases it was confirmed by demonstrating the acarus microscopically in scrapings from a burrow, and in case 6 the acarus was seen transected in the haematoxylin and eosin preparation. Four patients had a family history of concurrent scabies or pruritus. In case 4 (M.R.), who was a widower, living alone, the diagnosis was most difficult as microscopical examination for acari in scrapings from the lesions was persistently negative.

Consequently, he was the most fully investigated from the point of view of a malignant lymphoid neoplasm. All investigations were negative. The clinical symptoms, distribution and appearances of the rash suggested the diagnosis of persistent nodular scabies.

As patients with scabies presenting in the conventional manner are rarely biopsied, there are few reports of its histology in the literature.

In 1889, Török reported histological changes in sections from lesions in patients with scabies. He described vascular dilatation and oedema, with increased mitoses in the cells of the vascular endothelium. He attributed these changes to the effects of a secretion from the parasite on the tissues. Dubreuilh (1893) and Unna (1896) agreed with Török's report, and in addition Unna stated that parasitic faecal scybala were always seen and thus the histologist could always diagnose scabies.

Heilesen (1946) biopsied lesions in thirty-five patients with scabies. He found dermal changes in eighteen of these. The most common abnormality (eleven cases) was 'round cell' infiltration which was occasionally perivascular. Three cases had an infiltrate composed of polymorphs and mononuclear cells and two had a leukocytic infiltrate, one with oedema and haemorrhage. In two cases biopsy specimens of papular lesions showed a 'round cell' infiltrate in the dermis. In two cases the histological features were regarded as 'non-specific'.

Schischa (1900) noted the presence of 'round cell' infiltration which he stated was a feature of the disease in children, and also of penile lesions in adults.

In the patients with persistent papular lesions after scabies described by Ayres & Anderson (1932), the dermal changes were found to be perivascular 'round cell' infiltrations in the mid to deep dermis. Histological examination of the nodules in Samman's (1963) single case revealed a perivascular infiltration of lymphocytes, mononuclear and reticulum cells.

Haber (1963), discussing Samman's case, noted that some patients with scabies developed haemorrhagic oedematous papules which might progress to a necrotic punched-out ulcer. On the penis these were sometimes called 'scabious chancres'. He thought that the histological picture was usually that of a marked perivascular inflammatory reaction which might penetrate deep into the dermis. It differed from the appearances of a foreign body reaction and did not resemble a response to a persistent insect bite. He postulated that there must be absorption of toxic material through the epidermis via intercellular spaces into the papillary body, thence by lymphatics into the dermis.

Grant & Keczkes (1964) recorded the case of an 11-week-old infant who after treatment for scabies developed yellowish-brown nodules on the periumbilical region and forearms. Histologically, lymphocytes and eosinophils were found, as were histiocytes, some of which were reported as being 'in mitosis.'

Berge & Krook (1967) described histological examinations carried out at intervals varying from 3 weeks to 4 months in five cases of persistent nodular scabies; in all the appearances were similar. The epidermis was normal. In the dermis, infiltrates of lymphocytes, reticulum cells, histiocytes and some eosinophils were present, mainly in clusters around the vessels and sweat glands.

In this laboratory, criteria for the histological diagnosis of cutaneous malignant lymphoid neoplasia include the presence of a pleomorphic dermal infiltrate consisting of histiocytes, reticulum cells, lymphocytes and plasma cells. Some cells should have hyperchromatic nuclei and others mitotic figures. The depth of this infiltrate in the dermis is significant. Any infiltrate in relation to or deep to the skin appendages is regarded as suspicious. To this picture may be added the finding of focal free haemorrhage in sites not related to biopsy trauma. Thus the quality as well as the quantity and depth of the dermal infiltrate must be considered when the question of possible malignant lymphoid neoplasia is raised.

In the present series the histological features in cases 1–4 were highly suggestive of lymphoid neoplasm on the basis of the criteria defined above. In view of the later clinical features and subsequent progress the remainder of the blocks of tissue from cases 1–4 was serially sectioned. In no instance were acari, ova or excreta demonstrated.

In case 5 the features suggested the possibility of an adverse drug reaction, although a lymphoid neoplasm could not confidently be excluded on purely histological grounds.

In the remaining case (case 6), the type and the extent of the dermal infiltrate was such that the possibility of a superimposed drug reaction was suggested. The case differed from the others in as much as an acarus was clearly demonstrated histologically. Moreover, the lesions in this patient cleared very rapidly compared with the others. As the patient had been receiving systemic antihistamines it may be that this histology is simply that of scabies plus an adverse drug reaction. On the other hand, it is possible that it represents the initial stage in the development of a similar histological picture to that of the previous patients.

We were given the opportunity to examine histological sections of the case reported by Dr Grant and Dr Keczkes, and in the absence of a clinical history felt that these could well have been reported as being suspicious of a malignant lymphoid neoplasm.

From a purely histological point of view, in the cases which showed only a pleomorphic dermal infiltrate, the possibility of pseudo-lymphomas, such as actinic reticuloid and lymphomatoid papulosis, must be considered. Such conditions can be excluded by the history of light sensitivity in the former and by the characteristic clinical course in the latter. The presence of lymphoid follicles in case 4 raised the possibility of Spiegler–Fendt sarcoid. This condition, however, characteristically shows fully developed germinal centres and a rim of eosinophils deep to the lymphocytes in the infiltrate. In addition, the clinical features of Spiegler–Fendt sarcoid were absent. The finding of a pleomorphic dermal infiltrate should always raise the possibility of a persistent insect bite, but in none of the cases was there any evidence of pseudo-epitheliomatous hyperplasia. We should like to emphasize that histological appearances in the absence of complete clinical details may be misleading. Indeed, even when clinical details are known the true diagnosis may ultimately be revealed only in the light of the subsequent progress of the patient and the response to therapy. We certainly do not agree with Unna (1896) that scabies is easy to diagnose histologically.

The mite in scabies does not appear to penetrate deeper than the keratin layer (Haber, 1963). Thus the reaction which extends so deeply in the dermis must be due to some mediator substance. Török (1889) suggested that there was a secretion from the parasite. Mellanby (1944) demonstrated that there was no reaction immediately surrounding the acarus but rather at the point where the mite had been 24 h previously. Even when the parasite was killed or removed, the reaction continued. Thus our inability to find an acarus even on ribboning-out some of the biopsy specimens need not be surprising.

An immune response of the delayed hypersensitivity type occurs in scabies (Burgess, 1973). Benjamini & Feingold (1970) described a similar response to bites by other anthropods. It may be that the epithelial cells of the basal cell layer are immunologically non-competent (Gaafar, 1972). If this is so, then the antigenic complex from the acarus must gain access to the dermis. Mellanby (1944) injected some extract of the mite of human volunteers. There was no response in persons who had never had scabies, nor in those infested for less than 3 months. However, in those infested for 6 months and over, a positive result was obtained. In spite of intensive efforts since then, it has not been possible to isolate arthropod antigens in chemically pure forms (Gaafar, 1972).

The purpose of this paper is to highlight the ominous histology that we have seen in persistent nodular scabies and thus alert clinicians investigating similar cases. It should be remembered, however, that a malignant lymphoid neoplasm and scabies are not mutually exclusive.

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Pharmacology and Treatment

Treatment of chronic urticaria with a proteinase (kallikrein) inhibitor

N.BEROVA, I.PETKOV AND V.C.ANDREEV

Department of Dermatology, Academy of Medicine, Sofia, Bulgaria

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SUMMARY

Results are reported of Trasylol treatment of fifty-two patients with various forms of urticaria. 100,000 anti-kallikrein units were given intravenously every other day, with 15–20 injections per course. An excellent therapeutic effect was obtained in 44% (twenty-three patients), and another 37% (nineteen patients) were greatly improved. Additional evidence of the objective therapeutic benefit was obtained in a double-blind study (twenty patients).

The most important factors in allergic shock are the increased permeability of the blood vessel walls and the proteolytic activity of the plasma. The development of these events depends on the presence of certain proteolytic enzymes, as well as on plasma kinins. The antigen–antibody complex specifically stimulates the plasma kinins, which represent important chemical mediators of the allergic reaction.

Three kinds of plasma kinins are of particular clinical importance, bradykinin (kinin 9), kallidin (kinin 10) and methonyl-bradykinin (kinin 11). They have almost identical pharmacological actions; they increase blood vessel permeability, stimulate leukocyte response, enhance tissue oedema and cause pain. The release of plasma kinins is a complex process which comes into effect through the activation of certain specific enzymes, kininogens, amongst which kallikrein is of particular importance.

Kallikrein exists in an inactive state as kallikreinogen in the pancreas, in the intestinal wall and in some of the body fluids (sweat, cerebrospinal fluid, urine and plasma).

The conversion of kallikreinogen into kallikrein is triggered by a variety of physical or chemical factors, including antigen–antibody complexes.

The existence of three natural kallikrein inhibitors is recognized; two of them are in the serum, the third is to be found in the lungs, the liver, the lymph nodes and the parotid gland. The third, extracted from cattle parotid glands, has been introduced into clinical practice under the name of Trasylol (Bayer).

The action of Trasylol may be summarized thus: it inhibits the spontaneous activation of the proteolytic enzymes (kallikrein, trypsin, haemotrypsin, plasmin); it suppresses the secretion of pancreatic enzymes and kininogenase in blood vessel walls, and in this way inhibits the release of kinins, an inevitable event in traumatic shock.

Favourable reports have appeared concerning the use of Trasylol in surgical practice. In dermatology it has been used in eczema, psoriasis (Steigleder, 1964), acne conglobata (Franceschini, 1970), and other conditions, but its efficacy has been described as inconclusive. In experimental studies, Kalaidjiev, Jankulova & Petrunov (1970) have observed inhibition of antibody formation.

In 1968 we started a clinical trial of Trasylol in different forms of chronic urticaria. In the meantime the communication of Juhlin & Michaëlsson (1969b) appeared. They treated ten patients with urticaria and angio-oedema. Improvement was observed in six of them. To the best of our knowledge there are no other reports on this subject.

PATIENTS AND METHODS

First trial

Fifty-two patients aged 20–60 were included in the clinical trial of Trasylol. They were subdivided as follows: chronic urticaria, thirty-eight patients; cold urticaria, five patients; acute urticaria, four patients; angio-oedema, five patients.

The distribution of patients does not represent the actual ratio of the different forms of urticaria in this country but reflects only the patients available for study. The majority of these patients had responded poorly or not at all to conventional treatment.

Second trial

In a further twenty patients with chronic urticaria a double-blind study was carried out. These patients were divided into two groups, each consisting of ten patients. The first group received Trasylol, the second group received a placebo in an identical ampoule (provided by Bayer). Subsequently a cross-over double-blind study was carried out on the second group; i.e. after assessment of the placebo effect the patients were given Trasylol without the knowledge of the patient or of the physician.

Trasylol was given in the form of intravenous injections, each containing 100,000 antikallikrein units, every other day for 30 to 45 days. The patients were followed up for a period ranging from 6 months to 2 years.

To assess the results of the treatment as objectively as possible, the spread and intensity of the lesions and pruritus were scored in each patient on an arbitrary 10 point scale. The response was calculated on this basis for each individual patient for a period of 6–24 months, and scores were assessed as follows: complete cure, less than 2·5 points out of 10; improvement, 2·5–7·5 points out of 10; failure, 7·5 points out of 10.

RESULTS

The results of the first trial are shown in Table 1. It is seen that over 80% of patients were categorized as completely cured or improved after Trasylol treatment.

TABLE 1. Results of first trial

Complete cure	23 (44%)
Improvement	19 (37%)
Failure	10 (19%)

The results of the double-blind second trial are shown in Table 2. They indicate that Trasylol is more effective than a placebo in a direct comparison, and that changing from a placebo to Trasylol in a crossed double-blind manner improved the complete cure rate from 0/10 to 7/10.

TABLE 2. Results of second trial (double-blind)

First group (Trasylol)	
Complete cure	6
Improvement	2
Failure	2
Second group (placebo)	
Complete cure	0
Improvement	5
Failure	5
Second group (placebo replaced by Trasylol)	
Complete cure	7
Improvement	2
Failure	1

The best results were obtained in patients with angio-oedema and in those who appeared to have an allergic disposition as judged by the history, skin testing and laboratory tests. The failures were mostly in cases with acute urticaria. In patients with chronic non-allergic urticaria or with cold urticaria the improvement was, as a rule, temporary. No patients complained of any significant side-effects, although some have been reported (Juhlin & Michaëlsson, 1969b, 1970; Bauer, Futterman & Dreiling, 1971).

DISCUSSION

These results show that in a significant proportion of patients Trasylol appears to be a valuable medicament in the treatment of chronic urticaria. The results are even more impressive if one takes into consideration that almost all of the patients had received all the usual forms of therapy, including corticosteroids, with only temporary improvement or none at all.

The mechanism of action of Trasylol is not completely understood. Its half-life is comparatively short (30–60 min), and 1 week after intravenous injection its tissue and serum levels are so low that it cannot be directly responsible for the therapeutic effect. Furthermore, there is evidence that Trasylol does not inhibit cell mediated immunity (Berova, Petkov & Andreev, 1972). In higher doses, however, Trasylol suppresses the formation of C_1-esterase and thus inactivates kallikrein precursors (Donaldson & Evans, 1963). There is no certain evidence that in chronic urticaria the level of the kinins is raised, nor that formation of their inhibitor is decreased. However, in patients with hereditary angio-oedema, the amount of plasma inhibitor of kallikrein is decreased. In addition, patients with chronic urticaria and hereditary angio-oedema show an increased sensitivity to the intradermal injection of kallikrein; it induces a considerably larger and more tender oedematous weal than in normal subjects (Juhlin & Michaëlsson, 1969a). It is probable that in chronic urticaria there are non-specific hereditary or acquired enzyme deficiencies, which are favourably influenced by the kallikrein inhibitor.

Activators and inhibitors of kallikrein also activate and inhibit the complement and plasmin systems. We have concentrated on the kallikrein system, but others studying urticaria have shown equal interest in complement (Klemperer, Donaldson & Rosen, 1968) and fibrinolysis (Haustein, 1969; Ryan, Nishioka & Dawber, 1971). Indeed, Trasylol, though commonly used as a kallikrein inhibitor, is a superior plasmin inhibitor (Ambrus, Ambrus & Sanne, 1972). In this respect the use of epsilon-aminocaproic acid (EACA) in the treatment of urticarias, and the studies on the effects of proteinase inhibitors on the inflammatory response must be taken into account (Bertelli, 1968). The reported side effects of Trasylol or EACA in the treatment of urticaria are difficult to interpret since in some cases they have resulted in a severe exacerbation of the disorder (anaphylactic response) even during the first treatment. We have met no such side effects.

Further experimental studies are required in order to explain the action of Trasylol in chronic urticaria.

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Halcinonide in the treatment of corticosteroid responsive dermatoses

EUGENE LEIBSOHN AND FILLMORE K.BAGATELL

Affiliated Dermatologists Ltd, Phoenix, Arizona, U.S.A.

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SUMMARY

Eighty-eight patients with bilateral lesions of atopic dermatitis, eczematous dermatitis, neurodermatitis or psoriasis were treated, without occlusion, with halcinonide and with betamethasone 17-valerate creams (0·1%) and evaluated in a double-blind, paired comparison study. In the forty-three psoriatic patients, halcinonide was superior in twenty-three, betamethasone 17-valerate was superior in four, both drugs were equally effective in fourteen, and neither drug was effective in two. In the evaluation of all eighty-eight patients, halcinonide was superior in forty-two, betamethasone 17-valerate was superior in eight, both drugs were equally effective in twenty-nine, and neither drug was effective in nine. The overall superiority of halcinonide to betamethasone 17-valerate was highly significant ($P < 0{\cdot}001$). Halcinonide exhibited its superiority each week of the 3-week study. There were no side effects with either drug. The substitution of a chlorine atom for a hydroxyl group in the 21-position of the triamcinolone acetonide molecule, and the use of a specifically formulated cream vehicle containing propylene glycol and water, have yielded an extremely active topical anti-inflammatory drug.

One of the most profound changes in the topical treatment of skin diseases has been the use of anti-inflammatory corticosteroids. Since the synthesis of the topically active fluorinated corticosteroid, triamcinolone acetonide, by Squibb, numerous modificatons of this basic compound have been tested and some have been marketed. The ability of topically applied corticosteroids to induce vasoconstriction was first observed by McKenzie & Stoughton (1962). This basic assay method and its modifications have been extremely useful in the evaluation of corticosteroids with potential clinical use (McKenzie & Stoughton, 1962; Stoughton, 1969).

For percutaneous absorption to occur, the corticosteroid first must pass through the barrier layer in the stratum corneum. It is well documented that particle size and vehicle composition may have a pronounced effect on such percutaneous absorption, as measured by the vasoconstriction test (Place, Velasquez & Burdick, 1970; Barrett *et al.*, 1965; Busse *et al.*, 1969; Portnoy, 1965; Sarkany *et al.*, 1965; Reinstein *et al.*, 1972; Burdick, 1971). Not only does micronization of a corticosteroid improve its percutaneous absorption, but the extent of this absorption is further enhanced when the vehicle permits the drug to be solubilized, dispersed, or adequately suspended (Barrett *et al.*, 1965; Portnoy, 1965). Studies with *in vitro* gel diffusion and human skin model systems, indicate that optimal corticosteroid release is also a function both of concentration and of low aqueous solubility (high partition coefficient) in the vehicle (Poulsen *et al.*, 1968; Ostrenga *et al.*, 1971a, 1971b). It is apparent also from these same studies that an optimal vehicle for one corticosteroid may be a poor vehicle for a different corticosteroid.

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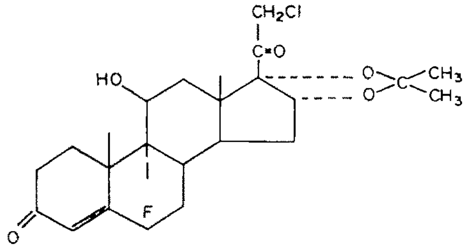
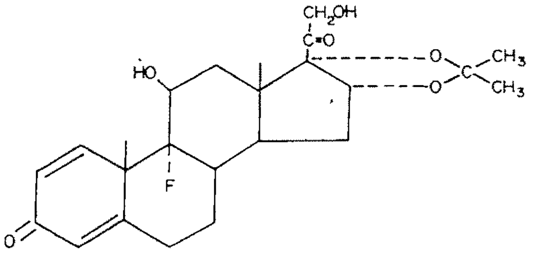
* Reprint requests to: Dr Eugene Leibsohn, 444 West Osborn Road, Phoenix, Arizona 85013, U.S.A.

Another factor in percutaneous absorption is the degree of hydration of the stratum corneum, which is normally hygroscopic in man (Shelmire, 1960). High concentrations of propylene glycol (75% or greater) tend to decrease the permeability to a corticosteroid of the stratum corneum barrier, and the decrease is proportional to the degree of dehydration of the barrier by the vehicle (Ostrenga *et al.*, 1971a, 1971b). There is little or no binding of the steroid within the barrier (Ostrenga *et al.*, 1971a, 1971b). Apparently, some degree of hydration of the epidermis is necessary for percutaneous absorption to occur (Ostrenga *et al.*, 1971a, 1971b; Cronin & Stoughton, 1962).



9α-fluoro-21-chloro-11β, 16α,17α,-trihydroxypregn-4-ene-3,20-dione, 16,17-acetonide

Halcinonide (SQ 18,566)



9α-fluoro-11β, 21-dihydroxy-16α,17α-(isopropylidenedioxy)-1,4-pregnadiene-3,20-dione

Triamcinolone acetonide

FIGURE 1. Chemical structures of halcinonide and triamcinolone acetonide.

It must be emphasized, however, that while considerably improved absorption may result from correct formulation, the prime consideration should be the selection of a compound with excellent topical activity. It has been determined, by means of two independent assays (McKenzie & Stoughton, 1962; Wells, 1957) that depend on the ability of potent topically active corticosteroids to cause vasoconstriction, that halcinonide meets these criteria (Bagatell, 1972). This compound differs from triamcinolone acetonide by substitution of a halogen (chlorine) for the hydroxyl group in position 21 and reduction of a double bond at positions 1 and 2 in ring A (Fig. 1). A specific vehicle was formulated for this corticosteroid, with a high content of propylene glycol, but containing sufficient water to maintain proper hydration of the stratum corneum.

METHOD AND MATERIALS

The therapeutic effects of halcinonide* and betamethasone 17-valerate (Valisone®, Schering) creams (0·1%) were compared in ninety Caucasoid patients (forty-four males, forty-six females). The age range of the patients was 8–72 years; the majority (94%) were adults.

The commercially available betamethasone 17-valerate cream formulation served as the control. Only patients with bilateral lesions of similar severity, persistence and aetiology were admitted to this double-blind, paired-comparison study. Forty-five patients had psoriasis, twenty-three eczematous dermatitis, thirteen lichen simplex and nine atopic dermatitis. Each patient was assigned a study number according to a table of assignment, and was provided at each weekly visit with one or two 15 g tubes of each drug, code-labelled with the study number. Assignment of test or control drug to left and right-sided lesions was randomized according to the patient's study number. The patients were evaluated at the initial visit for suitability and they returned at weekly intervals for follow-up treatment. A maximum study period of 3 weeks was allowed. Each patient applied both creams in a similar fashion in the morning, afternoon and evening (i.e. three times daily). The patients were instructed to apply to the left-sided lesions a designated cream containing corticosteroid, liberally and without occlusion, then to wash their hands before applying to the right-sided lesions, in a similar manner, the other designated cream. The use of other corticosteroids, antihistamines and antipruritics was prohibited during the treatment period.

Two patients failed to return after the first visit. Six patients were evaluated only at the end of week 1, and twenty-four were evaluated after weeks 1 and 2. Sixty patients were evaluated weekly for the 3-week treatment period (except for one patient who was not evaluated for week 2). Therapy ceased in eighteen patients when sufficient clinical remission had been obtained before the end of the suggested treatment period.

Objective criteria for judging therapeutic response included decrease of lesion size, as well as concomitant reduction of erythema, oedema, transudation, lichenification or scaling. Subjective criteria reported by the patient included relief of pruritus or pain, or of both. In rating the overall therapeutic response during treatment, the following factors were taken into account: (a) rapidity of onset of any therapeutic response; (b) maximum degree of clearing of the lesions; and (c) maintenance of any response during the period of treatment. The patient was questioned and examined for the occurrence of any local irritation or other type of side effect. All statistical analyses were performed by the method of McNemar (1947). Any '*P*' value less than 0·05 indicated a statistically significant difference between the two test compounds. A '*P*' value greater than 0·05 indicated that any apparent difference between the test compounds could have been due to chance.

RESULTS

Two patients (one male with atopic dermatitis and one female with lichen simplex) did not return after the first visit and were, therfore, not evaluated. The overall objective comparative clinical responses at the final visit for each individual skin disease and for all diagnoses combined are listed in Table 1. When the difference in efficacy between the drugs was slight, it was shown as 'slight superiority'. When this difference was marked, it was indicated as 'marked superiority'. For all diagnoses combined, halcinonide showed a statistically significant superiority to betamethasone 17-valerate. In the two diagnostic categories containing more than twenty patients (psoriasis and eczematous dermatitis), halcinonide was significantly superior to betamethasone 17-valerate cream.

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* Halcinonide (chlorodesoxy, dihydrotriamcinolone acetonide, SQ 18, 566) cream was supplied by The Squibb Institute for Medical Research, Princeton, New Jersey 08540, U.S.A.

Finally, for each of the four disease categories studied, the results favoured the former corticosteroid cream.

In the final evaluation for all diagnosis, halcinonide was superior in forty-two patients, betamethasone 17-valerate was superior in eight, both drugs were equal in twenty-nine and neither drug was effective in nine.

TABLE 1. Overall objective comparative clinical responses

Diagnosis	Halcinonide		Betamethasone 17-valerate		Equal	Neither drug effective	Total	*P* value	Lost to follow-up
	MS*	SS†	MS*	SS†					
Atopic dermatitis	1	3	0	2	2	0	8	NS‡	1
Eczematous dermatitis	2	7	0	1	8	5	23	$P<0{\cdot}01$	0
Neurodermatitis	1	4	0	1	4	2	12	NS	1
Psoriasis	10	14	0	4	15	2	45	$P<0{\cdot}001$	0
All diagnoses	14	28	0	8	29	9	88	$P<0{\cdot}001$	2

* MS, markedly superior; † SS, slightly superior; ‡ NS, not significant.

TABLE 2. Objective comparative responses by week

Diagnosis	Week	Halcinonide		Betamethasone 17-valerate		Equal	Neither drug effective	*P* value	Lost to follow-up
		MS*	SS†	MS*	SS†				
Atopic dermatitis	1	1	3	0	2	2	0	NS‡	1
	2	0	4	0	2	2	0	NS	
	3	0	3	0	2	1	0	NS	
Eczematous dermatitis	1	2	7	0	1	8	5	$P<0{\cdot}01$	0
	2	2	7	0	1	8	1	$P<0{\cdot}01$	
	3	0	3	0	1	4	0	NS	
Neurodermatitis	1	1	4	0	1	4	2	NS	1
	2	1	3	0	1	5	0	NS	
	3	0	3	1	0	4	0	NS	
Psoriasis	1	9	14	0	4	16	2	$P<0{\cdot}001$	0
	2	10	14	0	4	14	2	$P<0{\cdot}001$	
	3	7	12	0	4	15	1	$P<0{\cdot}001$	
All diagnoses	1	13	28	0	8	30	9	$P<0{\cdot}001$	2
	2	13	28	0	8	29	3	$P<0{\cdot}001$	
	3	7	21	0	7	24	1	$P<0{\cdot}001$	

* MS, markedly superior; † SS, slighly superior; ‡ NS, not significant.

The comparative responses at the end of each week of treatment are listed in Table 2. The apparent superiority of halcinonide cream to betamethasone 17-valerate cream was maintained each week in those diseases (psoriasis and eczematous dermatitis) where more than twenty patients were treated. This finding is contrary to the usual experience with potent fluorinated corticosteroids employed as topical anti-inflammatory drugs, where the apparent differences between preparations tend to disappear with time.

The overall evaluations of therapeutic responses, for all diagnoses, to each drug independently and for each of the individual diseases studied are summarized in Table 3. When all the patients were evaluated, the excellent and good responses combined comprised 74% of the total for halcinonide, and 53% of those for betamethasone 17-valerate. For psoriatic patients, the corresponding percentages for halcinonide and betamethasone 17-valerate were 81 and 60. The same pattern was observed for atopic dermatitis, eczematous dermatitis and lichen simplex. Again, the superiority of halcinonide to betamethasone 17-valerate is apparent. There were no side effects observed in this study for either drug.

TABLE 3. Overall evaluation of therapeutic response

	Excellent		Good		Fair		Poor	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Atopic dermatitis								
Halcinonide	1	13	4	50	3	27	0	0
Valisone*	1	13	2	25	5	62	0	0
Eczematous dermatitis								
Halcinonide	4	17	10	44	4	17	5	22
Valisone	0	0	10	44	8	34	5	22
Neurodermatitis								
Halcinonide	2	17	7	57	1	8	2	17
Valisone	0	0	6	50	4	33	2	17
Psoriasis								
Halcinonide	15	35	20	46	6	14	2	5
Valisone	5	12	21	48	15	35	2	5
All diagnoses								
Halcinonide	23	26	42	48	14	16	9	10
Valisone	6	7	41	46	32	37	9	10

* Valisone is betamethasone 17-valerate.

DISCUSSION

Halcinonide, a new corticosteroid, appears to represent a significant advance in dermatological therapy, especially for the treatment of psoriasis and inflammatory diseases of the skin.

The response obtained to therapy probably results from the potency of the corticosteroid and its appropriate vehicle. Introduction of a halogen atom at the apparently important position 21 of the triamcinolone acetonide molecule results in a very effective corticosteroid for topical therapy.

The mode of action of the glucocorticosteroids at the tissue level is still being investigated; no single biochemical explanation has yet been accepted. It is known that the fibroblasts are extremely sensitive to the effects of anti-inflammatory corticosteroids, both *in vivo* and *in vitro* (Dougherty, Berliner & Berliner, 1961). Berliner, Gallegos & Schneebeli (1961) have demonstrated morphological changes induced in tissue-culture fibroblasts by anti-inflammatory glucocorticosteroids. Cline & Nelson (1966) suggested that the anti-inflammatory action of cortisol may be due to its inhibition of the release of plasma kinins. The resultant reduction in the activated kallikreins would account for the anti-inflammatory process, *vis-à-vis*, dilatation of capillaries, development of oedema, deposition of fibrin, and migration of granulocytes into the inflamed area. Another theory that has gained some support is that the corticosteroids stabilize the lysosomal membranes with consequent reduction of the release of irritating hydrolytic enzymes (Rogers, 1964).

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Dapsone acetylation in dermatitis herpetiformis

G.A.ELLARD, PATRICIA T.GAMMON, J.A.SAVIN*
AND R.S.-H.TAN†

MRC Unit for Laboratory Studies of Tuberculosis, Royal Postgraduate Medical School, London, W.12, and St Thomas' Hospital, London, S.E.1

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SUMMARY

The metabolism of dapsone was studied in ten patients with proven dermatitis herpetiformis. Seven of the patients, who were slow acetylators of sulphadimidine, also acetylated dapsone slowly. Three of the patients were rapid acetylators of both drugs. The control of symptoms did not seem to be related to the plasma concentrations of dapsone or of its metabolite monoacetyldapsone. Haematological side effects, however, were related to plasma dapsone concentrations.

Despite the fact that dapsone (4,4′-diaminodiphenyl sulphone, DDS) has been used for over 20 years to control dermatitis herpetiformis, it is not understood why widely varying doses are needed to suppress the lesions of different patients. Previous studies have shown that individuals can be classified into two sharply distinct phenotypes according to the rates at which they acetylate certain drugs. Recently, Gelber *et al.* (1971), and Peters *et al.* (1972) have demonstrated that DDS, like isoniazid, sulphadimidine and certain other hydrazides and sulphonamides, is also acetylated polymorphically and that the plasma concentrations of 4-amino-4′-acetamidodiphenyl sulphone (MADDS) in rapid acetylators are considerably higher than in those of slow acetylators given the same dose of DDS. Unlike the acetylated derivatives of these other drugs, however, MADDS is de-acetylated quickly in man and constant ratios of MADDS to DDS are established in the plasma. The possibility was envisaged that if the symptoms of dermatitis herpetiformis were controlled by the metabolite MADDS, rather than by the administered DDS, rapid acetylators might require smaller doses of DDS to control their symptoms than slow acetylators. This paper describes a study of the acetylation of DDS by a small group of patients with dermatitis herpetiformis, to determine the plasma concentrations of DDS and MADDS that suppressed their symptoms, and the effect of the compounds upon the red blood cells.

METHODS

Ten out-patients with dermatitis herpetiformis, whose diagnosis had been confirmed by skin biopsy and by response to DDS, were included in the study. These patients were being treated with 50–300 mg DDS per day, the smallest doses required to control their symptoms. On the day before the study the patients were requested to take their DDS as a single daily dose at 10 a.m. On the day of the study, a blood sample was collected (0 h) at 10 a.m. from each patient. Immediately afterwards the usual daily dose of DDS was given, again as a single dose, together with a dose of approximately 11 mg/kg of sulphadimidine (dimethylsulphapyrimidine, sulphamethazine), according to the dosage schedule II described by Evans (1969). A light meal was taken 2 h later. Further blood samples were collected at 3 and 6 h together with a 5–6 h urine sample.

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* Present address: Department of Dermatology, Royal Infirmary, Edinburgh.

† Present address: Department of Dermatology, Westminster Hospital, London, S.W.1.

Plasma concentrations of DDS and of MADDS were determined fluorometrically (Ellard & Gammon, 1969). Evidence as to the identity of the compounds being measured was obtained by measuring the fluorescence of the ethyl acetate extracts at three wavelengths (298/345, 298/420 and 295/324 nm, respectively). The fluorescence characteristics of the ethyl acetate extracts used to determine the concentrations of DDS were identical to those of DDS. Before calculating the MADDS concentration, allowance was made for the fluorescence of the proportion of DDS not removed by extraction into 1·2 N HCl.

With but one exception, the corrected fluorescence of the extracts was like that of MADDS. Specificity studies demonstrated that neither sulphadimidine nor acetylsulphadimidine interfered significantly at the concentrations encountered in this study. Concentrations of free sulphadimidine and of acid-hydrolysable (free plus acetylated) sulphadimidine in plasma and urine were determined by a modification (Varley, 1962) of the Bratton & Marshall (1939) procedure. The plasma results obtained were corrected for the contributions due to DDS and MADDS as determined fluorometrically. Determination of the concentrations of acid-labile DDS in the urine samples (Ellard & Gammon, 1969) demonstrated that the urinary concentrations of DDS and its metabolites were too low to affect the classification of the acetylator status of the patients using sulphadimidine.

The concentrations of met haemoglobin and total denatured protein (including sulphhaemoglobin) in the 0 h blood samples (before the doses of sulphadimidine were given) were estimated by the method of Evelyn & Malloy (1938) with modifications as suggested by Van Kampen & Zijlstra (1965). Red blood cells were examined for Heinz bodies after staining with 1% methyl violet.

RESULTS AND DISCUSSION

The ten dermatitis herpetiformis patients were unequivocally classified as seven slow and three rapid acetylators, from the percentage of sulphadimidine acetylated in the plasma at 6 h (slow acetylators 13–35%, rapid acetylators 64–77%), or in the urine from 5–6 h (slow acetylators 36–60%; rapid acetylators 77–81%). The results are similar to those obtained previously using this method (Evans, 1969; Viznerova, Slavikova & Ellard, 1973).

The plasma concentrations of DDS and of MADDS, and the ratios of MADDS to DDS in the plasma are summarized in Table 1. The results confirmed the polymorphic acetylation of DDS demonstrated by Gelber *et al.* (1971) and by Peters *et al.* (1972). Thus, the ratios of MADDS to DDS in the seven slow acetylators were 0·15 or less, while those in the three rapid acetylators were 0·27 or more, and these ratios did not change with time, indicating that equilibrium between polymorphic acetylation and monomorphic de-acetylation was rapidly achieved. The plasma concentrations of free and of acid-hydrolysable Bratton & Marshall positive compounds at 0 h (before the dose of sulphadimidine was given) were identical to those expected on the basis of the fluorometrically determined concentrations of DDS and MADDS. These results confirm the findings of Alexander *et al.* (1970) and of Peters *et al.* (1972), and indicate that MADDS is the only major diazotisable metabolite of DDS found in the plasma. The results obtained demonstrated that the doses of DDS (which varied over five-fold) controlling the symptoms of the patients were associated with DDS plasma concentrations that varied

TABLE 1. Plasma concentrations of DDS and MADDS in relation to acetylator phenotype

Subject	Acetylator* phenotype	Dose DDS (mg/kg)	DDS concentrations (μg/ml)			MADDS concentrations (μg/ml)			Ratios MADDS/DDS		
			0 h	3 h	6 h	0 h	3 h	6 h	0 h	3 h	6 h
1	Slow	0·77	0·15	0·49	0·58	<0·10†	<0·10	<0·10	—	—	—
2	Slow	0·78	0·99	1·89	1·56	0·15	0·16	0·22	0·15	0·08	0·14
3	Slow	1·45	1·37	2·72	2·58	0·18	0·35	0·30	0·13	0·13	0·12
4	Slow	1·68	1·71	2·76	2·70	0·21	0·31	0·25	0·12	0·11	0·09
5	Slow	1·84	3·97	4·40	4·40	0·39	0·67	0·55	0·10	0·15	0·13
6	Slow	2·60	1·77	3·54	3·59	0·18	0·32	0·28	0·10	0·09	0·08
7	Slow	5·08	0·12	2·37	1·93	<0·16‡	0·18	0·29	—	0·08	0·15
8	Rapid	1·02	3·75	3·75	4·46	1·38	1·54	1·33	0·37	0·41	0·30
9	Rapid	1·56	—§	1·24	1·06	0·46	0·29	0·30	—	0·37	0·27**
10	Rapid	2·75	5·90	7·44	7·28	4·37	4·83	4·59	0·75	0·65	0·63

* Determined using sulphadimidine (see text).

† Below the lowest concentration measurable by the fluorometric method employed.

‡ Fluorescence characteristics of extract unlike that of MADDS.

§ Patient took normal daily dose of DDS 3 h before start of test instead of 24 h as instructed. Only sulphadimidine was given at time zero.

** 9 h MADDS/DDS ratio 0·30.

from about 0·5 μg/ml to greater than 5 μg/ml. The concomitant MADDS plasma concentrations ranged from less than 0·1 μg/ml to nearly 5 μg/ml. Thus, the control of dermatitis herpetiformis does not appear to be directly related to the plasma concentrations of either DDS or MADDS.

The haematological side-effects were found to be correlated better with peak DDS plasma concentrations ($P = 0{\cdot}04$ for methaemoglobin and denatured protein; $P = 0{\cdot}003$ for Heinz bodies) than to the daily mg/kg dose of DDS being taken ($P > 0{\cdot}3$) (Table 2). The high levels of total denatured proteins

TABLE 2. Haematological findings in relation to DDS plasma concentrations and dosage

Peak* plasma concentrations DDS (μg/ml)	Daily dose DDS (mg/kg)	Subject	Acetylator phenotype	Methaemoglobin (%)	Total denatured protein (%)	Heinz bodies†
0·54	0·77	1	Slow	0	0	0
1·13	1·56	9	Rapid	0	0	1+
1·73	0·78	2	Slow	0	1·9	Not done
2·15	5·08	7	Slow	0	0	0
2·65	1·45	3	Slow	0·8	4·8‡	2+‡
2·73	1·68	4	Slow	0	0	1+
3·57	2·60	6	Slow	0	4·9	1+
4·11	1·02	8	Rapid	3·5	9·8	2+
4·40	1·84	5	Slow	0	4·0	1+
7·36	2·75	10	Rapid	2·1	4·4	3+

* 0, none; 1+, some; 2+, many; 3+, many large.

† Mean 3 and 6 h values.

‡ Patient with spleen removed for hereditary spherocytosis.

and Heinz bodies in subject 3 probably resulted from previous splenectomy for hereditary spheorcytosis (Selwyn, 1955). Recent reports indicate that methaemoglobin and Heinz body formation are probably due to N-hydroxylated DDS rather than to DDS or MADDS (Cucinell, Israili & Dayton, 1972; Glader & Conrad, 1973; Kramer, Glader & Li, 1972; Scott & Rasbridge, 1973; Tabarelli & Uehleke, 1972), and it is likely that plasma concentrations of this metabolite would parallel those of DDS.

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Successful treatment of chromoblastomycosis with 5-fluorocytosine

WARWICK L. MORISON, BRUCE CONNOR AND
YVONNE CLAYTON*

Department of Dermatology, St Thomas' Hospital, London, and *Department of Medical Mycology,
St John's Hospital for Diseases of the Skin, London

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SUMMARY

A case of chromoblastomycosis was successfully treated by oral and topical 5-fluorocytosine. Progress of treatment was monitored histologically, by culture of scrapings, and by estimation of blood levels of the drug.

A variety of therapies has been used in the treatment of chromoblastomycosis. Recently, several authors have reported the successful use of a relatively new fungicidal agent, 5-fluorocytosine (5-FC). The following is a report of another case successfully treated with this drug, and this we believe is the first reported experience from England.

CASE REPORT

The patient, a 17-year-old West Indian student, had first noticed a lesion on his right leg 4 years previously, since when it had progressively increased in size. Local pruritus was the only complaint, there being no bleeding or episodes of secondary infection. His general health had remained unimpaired. He was born in a rural area of Jamaica, migrating to England 6 months prior to his first presentation for medical advice. There was no family history of similar disorders, and the patient had never been engaged in agriculture or gardening.

Examination revealed an almost continuous lesion over the lateral and anterior aspects of the right leg, composed of multiple hyperpigmented papules and plaques 0·2–2 cm in diameter, with five tender verrucous nodules 1–4 cm in diameter (Fig. 1). A small hyperpigmented nodule 0·5 cm in diameter was present on the dorsum of the right wrist. General examination was normal.

Histological examination of a biopsy from a verrucous nodule on the leg, and excision biopsy of the wrist lesion, showed the typical features of chromoblastomycosis (Fig. 2). In haematoxylin and eosin stained sections, pigmented spores were seen in and around giant cells, with a granulomatous infiltrate in the dermis, and pseudo-epitheliomatous hyperplasia of the epidermis. Ground-up biopsy tissue was cultured on Sabouraud's dextrose agar, and after 2 weeks' incubation at 28°C, colonies of *Phialophora pedrosoi* developed. The sensitivity to the strain to 5-fluorocytosine was assessed in Casmino acid broth medium (Shadomy, 1969). After 7 days incubation at 28°C, the concentration of the drug required to inhibit growth was found to be 12·5 μg/ml.

Haemoglobin, white cell count, ESR, chest X-ray, liver function tests and examination of the urine were all within normal limits.

TREATMENT SCHEDULE

5-FC was administered by both the oral and topical routes for a period of 6 months. Orally, the drug was given daily in three divided doses, the total daily dose being: 100 mg/kg for 2 weeks; 200 mg/kg for 2 weeks; and then 150 mg/kg for 5 months.

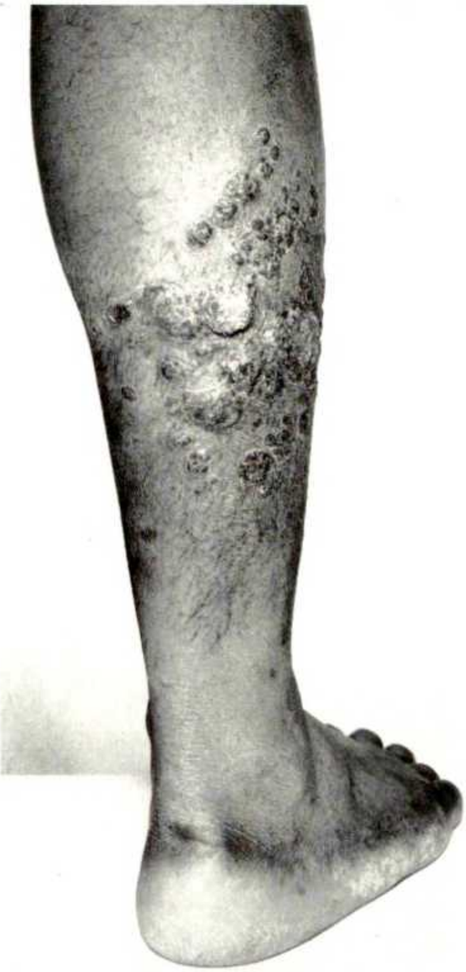

FIGURE 1. Chromoblastomycosis: clinical appearance before treatment.

Topically, 10% 5-FC ointment was applied under polythene occlusion daily. For the first month the patient was treated in hospital and thereafter it was supervised at regular out-patient attendances.

Serum levels of the drug were estimated on blood samples taken 4 h after oral administration of 5-FC, using a diffusion assay technique. The medium employed was yeast nitrogen base agar, and the test organism was a strain of *Candida pseudotropicalis*. A standard curve was prepared for the dilution of 5-FC used, plotting zone size in mm against the logarithm of the concentrations. The concentration of the drug in the patient's serum then being calculated from the graph.

In addition to clinical assessment, response to treatment was followed in two ways: weekly surface scrapings from the lesions were cultured until negative for organisms, and skin biopsies were taken monthly and were examined histologically and by culture until there was no evidence of infection. To check for possible drug toxicity, a full blood count, liver function tests, blood urea and microscopy of urine were carried out, at first twice weekly, later weekly and finally monthly.

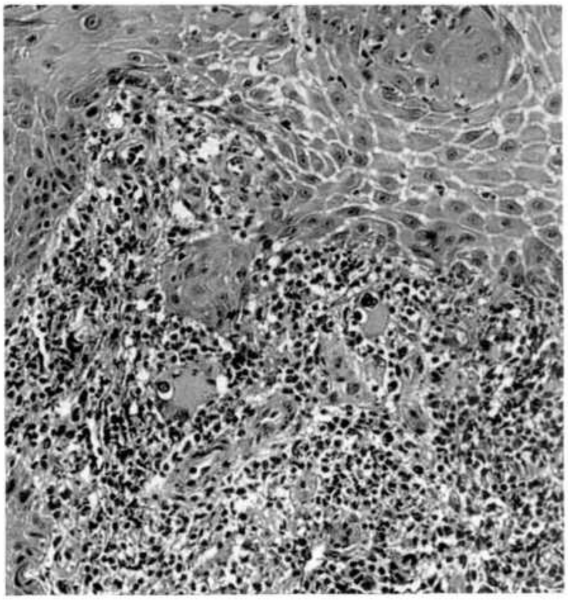

FIGURE 2. Low power view of biopsy showing pigmented spores in giant cells.

RESULTS OF TREATMENT

Clinically, from the end of the first month of treatment there was a progressive improvement: all lesions appeared flatter and less indurated. After 6 months of treatment, soft scar tissue, showing depigmentation in some areas, was the only remaining evidence of the previous infection (Fig. 3). There has been no sign of recurrence 6 months after completing the treatment. Culture of skin scrapings and biopsy material became negative for organisms after 1 month of treatment. Histological examination at that time revealed a considerable reduction in the number of organisms, and the few present appeared to be damaged and non-viable. After 2 months of treatment no organisms could be found on biopsy, and all subsequent biopsies remained negative for organisms, whilst the verrucosity and granulomatous reaction showed progressive resolution.

The dosage and duration of treatment, with resultant serum levels of 5-FC, are given in Table 1. An attempt was made to measure the tissue level of 5-FC in a biopsy specimen. The previously weighed tissue was ground-up in a known volume of distilled water, and an assay performed by the method described for determining serum levels. It was calculated that the specimen contained $<0{\cdot}5\ \mu g$ of 5-FC/g of tissue; at the time of measurement, the serum level was 72 μg/ml. Symptomatically, the patient remained free of side effects throughout the treatment. 2 weeks after the dose of 5-FC was raised to 200 mg/kg/day, there was a transient decrease in the total white cell count from 5200/mm^3

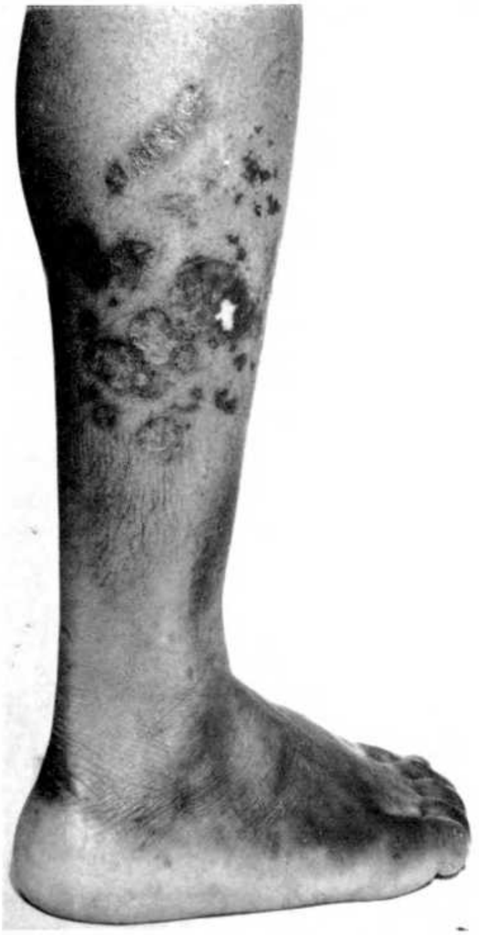

FIGURE 3. Chromoblastomycosis: clinical appearance after 6 months' treatment.

TABLE 1.

Dose (mg/kg/day)	Duration of dosage schedule	Resultant serum level (μg/ml)
100	2 weeks	20–35
200	2 weeks	55–72
150	5 months	30–42

to 3400/mm^3 with a normal differential count. The dose of 5-FC was reduced to 150 mg/kg/day and the next and all subsequent white cell counts were normal.

DISCUSSION

The patient presented a difficult management problem as there is no entirely successful treatment of chromoblastomycosis. Surgical excision or curettage and cautery are the treatments of choice for small lesions, but have a high recurrence rate when used on large areas. Of the many drugs tried, intralesional amphotericin B has received the most thorough evaluation but it appeared to be curative only in small lesions, which could in any case have been excised (Whiting, 1967). More recently, thiabendazole has achieved some favour but improvement without cure has been a common finding, severe side-effects in occur some cases (Bayles, 1971).

A number of favourable reports on the use of 5-FC in chromoblastomycosis have recently been published (Lopes, 1969, 1970, 1971; Itani, 1970). The largest trial involved ten patients, all of whom were cleared of the infection (Magalhaes & Meyer, 1970). No significant side effects have been reported from the use of 5-FC in chromoblastomycosis. When used in cryptococcosis, candidiasis and other fungal infections, untoward effects have been noted, especially among severely ill patients, but in such cases it is difficult to assess whether the drug or the underlying disease was responsible. In our patient the drug was well tolerated throughout the course of treatment, the only side effect being a transient fall in the total white cell count when the serum level was of the order of 50–52 mg/ml. Leukopenia has been reported in fifteen of 247 patients who have received 5–FC (Roche Products Ltd, 1972).

The drug has an interesting mechanism of action; susceptible fungi deaminate 5-FC to 5-fluorouracil intracellularly, which is then toxic by enzyme interference being incorporated into ribonucleic acids. 5-FC is not metabolized in human tissues due to the absence of the deaminase.

This patient has shown an excellent response to treatment, and it would appear that 5-FC is a very satisfactory agent in the treatment of chromoblastomycosis.

ACKNOWLEDGMENTS

We wish to thank Dr H.J.Wallace for permission to publish details of his patient. This patient was presented at the Royal Society of Medicine in April 1972 and a case report was published in the Proceedings of the Society (Morison, 1973). 5-FC tablets and ointment were kindly supplied by Roche Products Ltd.

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Pellagra, serotonin and 5-HIAA

J. STRATIGOS, A. KATSAMBAS AND P. GALANOPOULOU

Departments of Dermatology, A. Sygros Hospital, and Experimental Pharmacology, School of Medicine,
University of Athens, Greece

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SUMMARY

In patients with pellagra, levels of serotonin in the serum and of 5-hydroxyindolecetic acid in the urine were consistently higher than in control subjects.

It has been well documented that pellagra is a clinical syndrome which arises from a cellular deficiency of niacin (Spies, Cooper & Blankenhorn, 1938; Duncan, 1965; Barrett-Connor, 1967; Smeenk & Hulsmans, 1969). It is also well known that tryptophan can be metabolized to nicotinic acid as well as to serotonin and to its final metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Udenfriend & Brodie, 1958; Sjoerdsma, 1959; Smeenk & Hulsmans, 1969), although the processes involved are complex and are not completely understood. We wondered whether the deficiency of nicotinic acid in pellagra patients might be correlated with changed levels of serotonin in the serum or of 5-HIAA in the urine.

PATIENTS AND METHODS

Serum serotonin (Waalkes, 1959) and urinary 5-HIAA (Arterbery & Conley, 1967) were determined in twenty patients with pellagra (eighteen males and two females), and in sixty controls (thirty males and thirty females). Their ages ranged from 20 to 70 years. Blood and urine were taken before any kind of treatment. None of the patients or of the controls was on a diet which is known to affect serum serotonin. Routine laboratory tests, including porphyrins and serum iron, were normal in all the subjects.

RESULTS

Subjects	Serum serotonin (mean, mg/ml)	Urinary 5-HIAA (mean, mg/24 h)
Pellagra (20)	0·23	17·45
Controls (60)	0·15	4·11

DISCUSSION

The mean values for serotonin and for 5-HIAA were significantly higher in pellagra patients than in the controls. Similar observations have been reported by Khatab *et al.* (1967) and by Stratigos *et al.* (1973). The deficiency of nicotinic acid in the patients may be regarded as beyond doubt. Thus the consistent increases in serum serotonin and in the end product (5-HIAA), suggest that in pellagra a redirection of tryptophan metabolism to serotonin and 5-HIAA takes place at the expense of protein and niacin synthesis. It is also possible that the photosensitization of the skin in pellagra patients is linked to the increased levels of serotinin and 5-HIAA.

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Case Reports

A case of optic atrophy possibly induced by quinoline in acrodermatitis enteropathica

ANTONIO GARCIA-PEREZ, CARMEN CASTRO, ANTONIO FRANCO AND RICARDO ESCRIBANO

Department of Dermatology, University of Salamanca, Fonsec 2, Salamanca, Spain

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SUMMARY

A 2-year-old girl with acrodermatitis enteropathica, developed optic atrophy during quinoline treatment (600 mg/day, later gradually increased to 1700 mg/day; a total dose of 280 g was given). The optic atrophy developed rapidly 9 months after the start of the quinoline treatment. The right eye was blinded and developed a convergent strabismus. Eight months after the quinoline had been stopped, the left eye began a slow and partial recovery of vision with an improvement in the appearance of the optic disc.

The quinolines, when used in high doses, control acrodermatitis enteropathica and produce a remission which lasts as long as the drug is administered (Dillaha, Lorincz & Avvik, 1958). Their pharmacological action remains unknown. It is often stated that the drug is scarcely absorbed and that treatment with it is, therefore, harmless.

In 1966, Berggren and Hansson reported the first of very few patients with optic atrophy due to quinolines.

CASE REPORT

Patient Y.G.P., female, was born on 16 January 1969, and breast fed for 3 months. At 6 months she began to have persistent and severe diarrhoea: no candida was isolated. When 12 months old, she developed a diffuse alopecia and lesions on the hands and feet. When she was first examined at the age of 17 months, she had fissured keratotic lesions on the palms and soles and paronychia of fingers and toes. Her weight and height were much below normal.

Treatment with iodochlorhydroxyquin was started (600 mg/day), and after 2 months the dose was gradually increased until a remission was achieved; this required doses of 1500–1700 mg daily. Treatment lasted 9 months. The total amount of quinoline administered was about 280 g.

After 9 months treatment, her parents noted a rapid deterioration of her visual capacity. The girl stumbled and fell, easily losing her sense of direction; after a few days she could distinguish only the shapes of objects such as simple toys, and at a very short distance. An ophthalmological examination revealed 'very pale optic discs with sharp edges' and a diagnosis of 'simple bilateral papillary atrophy

TABLE 1. Reports of neuropathy associated with quinoline administration

Case no.	Author	Age (years)	Daily dosage (g)	Duration of treatment	Total dosage (g)	Associated neuropathy
1	Berggren & Hansson (1966)	5	1·2–2	11 months	400–600	No
2	Etheridge & Stewart (1966)	3½	3·6	2 years	2600	No
3	Berggren & Hansson (1968)	47	0·6–0·8	2 years	500	No
4	Strandvik & Zetterström (1968)	12	1·5	27 days	300	Yes
5	Kaeser & Wuthrich (1970)	58	0·750–2·250	8 years	3900	Yes
6	Abeledo Mezquita *et al.* (1971)	2	0·6–1	2 years	570	No
7	Osterman (1971)	24	1·5	2 months	90	Yes
8	Van Balen (1971)	$3\frac{2}{12}$	?	3 years 4 months	1279	No
9	Boe & Egge (1971)	2½	0·750	8 months	180	No
10	Boe & Egge (1971)	12	0·500–0·750	9 years	1600	No
11	Billson, Reich & Hopkins (1972)	18	2	9 months	540	No
12	Billson *et al.* (1972)	75	1	2 years	700	Yes
13	Bron *et al.* (1972)	37	0·750	6 years	2100	Yes
14	Cambier *et al.* (1972)	66	1·2	18 months	648	Yes
15	Drukker & Lindenburg (1972)	1½	0·375–0·5	?	12	No
16	Pedersen & Christiansen (1972)	41	1·250–1·5	6 years 9 months	3650	Yes
17	Present case (1972)	2	0·6–1·7	9 months	280	No

with defective vision' was made. The medication was stopped; the diarrhoea recurred, but was controllable by dietetic measures.

The optic disc and the visual acuity of the left eye began to show signs of recovery 13 months after stopping the quinoline. But the papillary atrophy of the right eye persisted and a convergent strabismus developed with absence of central vision and with eccentric fixation. Over the next year the left eye continued to improve; the right eye went blind.

Clinical and experimental studies as early as 1932 demonstrated that a very variable part of the orally administered drug is excreted in the urine as sulphates and glucuronates (quoted in Berggren & Hansson, 1968) but reported cases of toxic optic neuritis due to quinolines are rare. We have been able to find only nineteen such cases, including our own. In seventeen of these cases, eight of them children, the daily dose and length of treatment were clearly specified, permitting an approximate calculation of the total amount of drug absorbed. These seventeen cases are summarized in Table 1. Six of the children had acrodermatitis enteropathica.

Most of the patients received high doses of quinolines for a long time, but there is no constant relationship between the optic atrophy and the daily or total dosage or both.

ACKNOWLEDGMENT

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Fibrosarcoma arising in long-standing mycosis fungoides

D.G.C.PRESBURY,* I.C.TALBOT AND W.F.KEALY†

Departments of Dermatology and Morbid Anatomy,
King's College Hospital, Denmark Hill, London, S.E.5

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SUMMARY

A patient suffering from mycosis fungoides is described, in whom a fibrosarcoma arose in the skin. No previous report of a connective tissue sarcoma occurring in a patient with mycosis fungoides has been traced.

Mycosis fungoides (MF) is a disease which passes through three stages. A pre-reticulotic stage precedes infiltration and tumour formation. Sometimes tumour formation occurs from the onset (*tumeur d'emblée* type), and in such patients it may be extremely difficult to distinguish this disease from reticulum cell sarcoma or lymphosarcoma presenting with skin tumours. The course of MF is usually protracted, with death due to an unassociated cause. However, proven MF may undergo change into a systemic lymphoma or leukaemia.

Out of 165 cases of biopsy-proven MF described by Cyr, Geokas & Worsley (1966), reticulum cell sarcoma developed in fourteen, lymphosarcoma in fifteen, and Hodgkin's disease in seven. 106 patients were judged to have died of their original disease. Five of those with reticulum cell sarcoma developed terminal monocytic leukaemia, and six with lymphosarcoma developed terminal lymphoblastic leukaemia.

Other reports have recorded the transition of MF to lymphoma or leukaemia in one or more cases (Fraser, 1925; Keim, 1929; Wile & Stiles, 1935; Combes & Bluefarb, 1941; Block *et al.*, 1963; Reed & Cummings, 1966).

One patient has been recorded in whom MF and Kaposi's haemorrhagic sarcoma occurred together (Lane & Greenwood, 1933). This association is interesting, as it has been suggested that Kaposi's sarcoma may be one of the lymphoid tumours (Willis, 1948).

We describe a patient who developed a fibrosarcoma in skin which for many years had been affected by MF. This change does not seem to have been reported before.

CASE REPORT

A 57-year-old man presented with a lesion on the extensor aspect of the right elbow. It had been noticed several months before, had increased in size during the preceding 6 weeks, and had bled slightly. He had had for 25 years a widespread skin eruption, previously thought to be psoriasis.

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* Present address: St John's Hospital for Diseases of the Skin, Lisle Street, London, W.C.2.

† Present address: Kingston Hospital, Kingston-upon-Thames, Surrey.

On examination, he had, over the lateral extensor aspect of the right elbow, a smooth, 5 cm nodule with a lobulated edge. It was attached to the skin, but not to the deep structures. His skin showed widespread areas of poikiloderma, and many infiltrated, scaly, erythematous plaques, with the appearance, over the abdomen and buttocks, of parakeratosis variegata. The extreme erythema may have been exaggerated by the prolonged use of topical fluorinated steroids. There was erosion of the right ala nasi with a pearly edge, suggesting a basal cell carcinoma. There was no lymphadenopathy. The liver and spleen were not palpable.

On the chest radiograph, a rounded opacity was noted above the posterior part of the left dome of the diaphragm. The significance of this finding was uncertain at this stage.

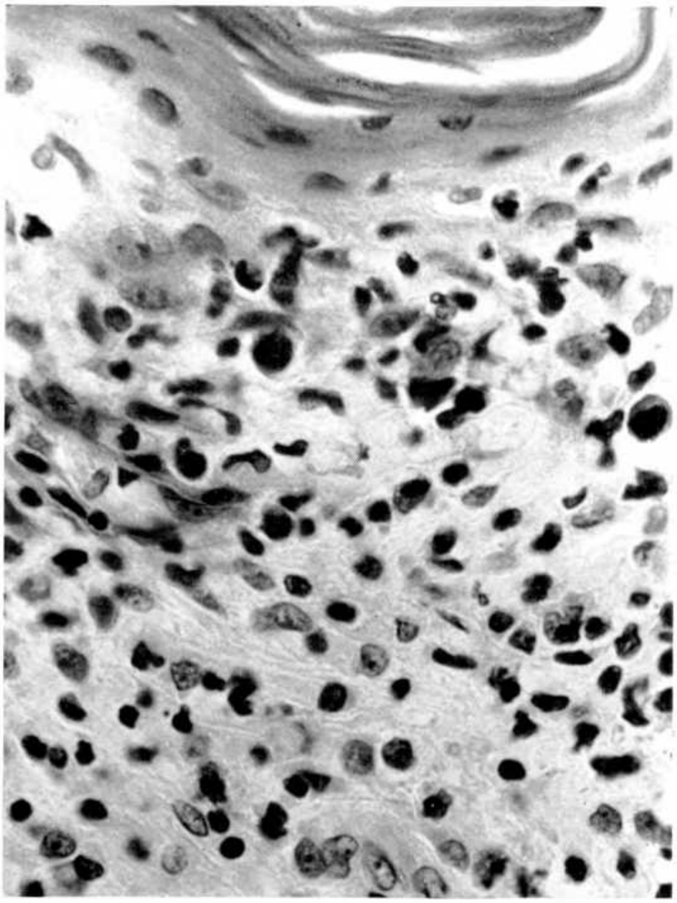

FIGURE 1. Biopsy of infiltrated, erythematous lesion showing infiltration of the epidermis by abnormal cells characteristic of mycosis fungoides (H & E, × 400).

Histological findings

Biopsies were originally taken from a plaque with the clinical appearance of MF and from the tumour of the elbow. The former showed an upper dermal infiltrate of mononuclear cells with large, pleomorphic, hyperchromatic nuclei. Similar cells had infiltrated the epidermis (Fig. 1) with occasional Pautrier abscess formation. This picture confirmed the diagnosis of MF.

The tumour of the elbow was composed of spindle cells arranged in whorls and bundles (Fig. 2). No epithelial differentiation was seen in the tumour, the periodic acid Schiff stain being negative, whilst the alcian blue stain for acid mucopolysaccharide was weakly positive. No myofibrils were seen after staining with phosphotungstic acid haematoxylin. In view of the large amount of reticulin present between the cells, the tumour was considered to be a fibrosarcoma.

Biopsy of the nasal lesion confirmed that it was a basal cell carcinoma.

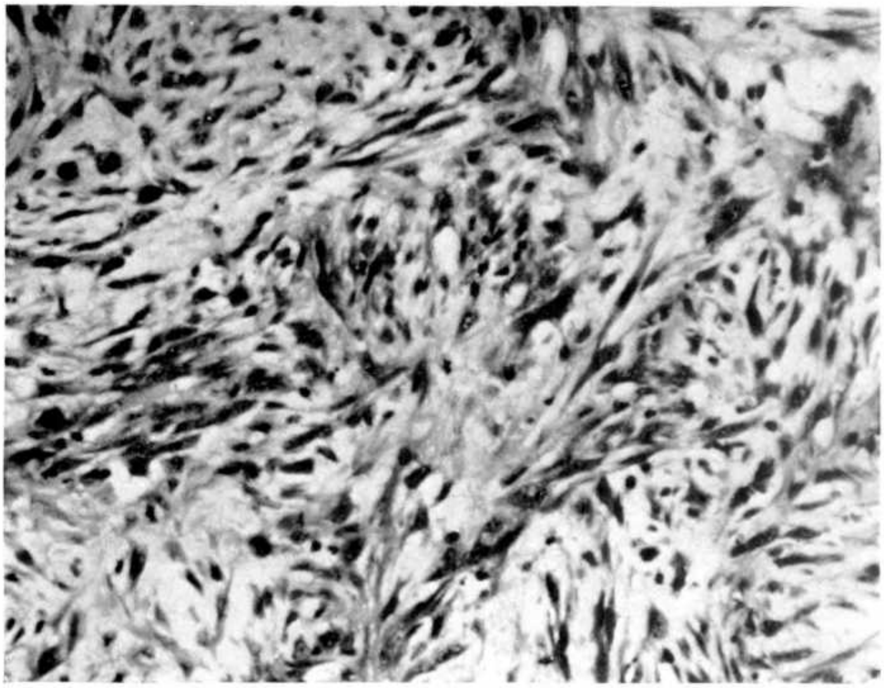

FIGURE 2. Biopsy of the tumour of the elbow showing the loosely-arranged, whorled spindle cells with large hyperchromatic nuclei, consistent with a soft tissue sarcoma (H & E, × 250).

Treatment and progress

The tumour was treated by radiotherapy. Initially the response was favourable, but within 2 months there was a recurrence, with two lesions at the original site. These were excised, but recurred again within a further 2 months. At this time no change was seen in the appearance of the chest radiograph. Nine months after he was first seen he had a fungating mass at the right elbow, with enlarged right epitrochlear and axillary glands. Multiple, rounded opacities were seen scattered throughout both lung fields on the chest radiograph. The appearance was consistent with secondary neoplasm. His condition deteriorated and he died 1 year after he had been first seen.

Autopsy findings

The whole of the skin was erythematous, with areas of plaque formation and small ulcers. The right ala nasi had been excised. There was an extensive healed scar on the lateral aspect of the right elbow, and on the medial side a soft mass, 6 cm × 5 cm, was attached to muscle. Numerous soft fleshy umbilicated tumour metastases were present in the lungs and pleurae, pericardium, and right kidney. The spleen and liver were slightly enlarged but contained no tumour. Histology of the skin showed

MF, and that of the mass and the metastases, showed a spindle cell sarcoma. Sections of the liver and spleen showed only congestive changes.

DISCUSSION

The development of a lymphoma in a patient with MF has been adequately documented, and is consistent with the concept of MF as one of the reticuloses. There have been several reports of carcinoma arising in patients with MF but their frequency has not suggested that this occurrence was more than coincidental (Skeer, 1943; Beerman & Thomas, 1946; Hynes & Wintrup, 1946; Gold, 1950; Bluefarb, Ihrke & Steinberg, 1952). We have, however, been unable to find any report of the concurrence of MF with a connective tissue tumour, arising in the skin, such as the fibrosarcoma which caused the death of the patient described here. Fibrosarcoma of the skin and subcutaneous tissue has been reported following radiotherapy, in lupus vulgaris and in burn scars (Gentele, 1951; Russell, 1959). This patient had none of these predisposing factors. Whilst the combination is almost certainly fortuitous, we believe that this case is unique.

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We are grateful to Mr A.J.Heriot for his permission to report this case.

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Macular amyloidosis simulating naevoid hyperpigmentation

MARTIN M.BLACK AND HOWARD I.MAIBACH

Department of Dermatology, University of California School of Medicine,
San Francisco, California, U.S.A.

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SUMMARY

A 17-year-old female is described who presented with an unusual form of macular amyloidosis which closely simulated naevoid hyperpigmentation. Electron microscopy was necessary to provide specific identification of the sparse deposits of amyloid.

Since Palitz & Peck described a macular form of cutaneous amyloidosis in 1952, the largest numbers of recorded cases of this particular type have originated from Central or South American countries (Porto, 1962; DiPrisco, Campo Aasen & Gomez, 1963; Cortés, 1959, 1969; Brownstein & Hashimoto, 1972). In recent years a number of communications have indicated that macular amyloidosis is not uncommon in the Middle East (Shanon & Sagher, 1970; Kurban *et al.*, 1971), Asia, and even Europe (Black & Wilson Jones, 1971). The usual presentation is as pruritic hyperpigmented lesions on the upper back, but at times the chest and extensor aspects of the extremities can be affected. A reticulate or 'rippled' pattern of pigmentation is often present (DiPrisco *et al.*, 1963; Kerdel-Vegas, 1967; Brownstein & Hashimoto, 1972), which has been emphasized as a characteristic and diagnostic feature of macular amyloidosis. However, atypical cases may also occur (Black & Wilson Jones, 1971; Black, 1972a). This communication describes and illustrates the clinical features of a girl who presented with an unusual variant of macular amyloidosis, which clinically presented as a type of naevoid hyperpigmentation.

CASE REPORT

L.H., a 17-year old Caucasoid girl (her maternal grandparents were Portuguese, but no close relatives came from Asia, Central or South America), first noticed the presence of irregular areas of brown pigmentation on the inner aspects of her left lower leg and ankle about 1 year previously. Since then she had become aware of similar pigmented areas on the left thigh and left lower abdomen. The pig-

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Reprint requests to: Howard I.Maibach, M.D., Department of Dermatology, 1096-HSE, University of California Medical Center, San Francisco, California 94122, U.S.A.

mented lesions had not progressed over a period of 6 months and were not pruritic. Her general health was excellent and there was no history of drug ingestion. Her mother suffered from allergic rhinitis, but there was no other family history of atopy.

Examination revealed hyperpigmented lesions, which were confined to the left half of the body, affecting parts of the medial aspect of the thigh, lower leg, ankle and also the lower abdomen. The lesions on the thigh and leg consisted of groups of confluent hyperpigmented macules, about 2 or 3 cm in diameter. However, the margins of the eruption were poorly defined; the edge of the pigmented macules tended to merge imperceptibly with clinically normal skin. The lesions on the left lower abdomen were clinically more conspicuous than those on the leg. Here, the hyperpigmentation was more marked in some of the macules and the lesions assumed an arcuate distribution (Fig. 1). In no area was a reticulate or 'rippled' pattern to the pigmentation noted.

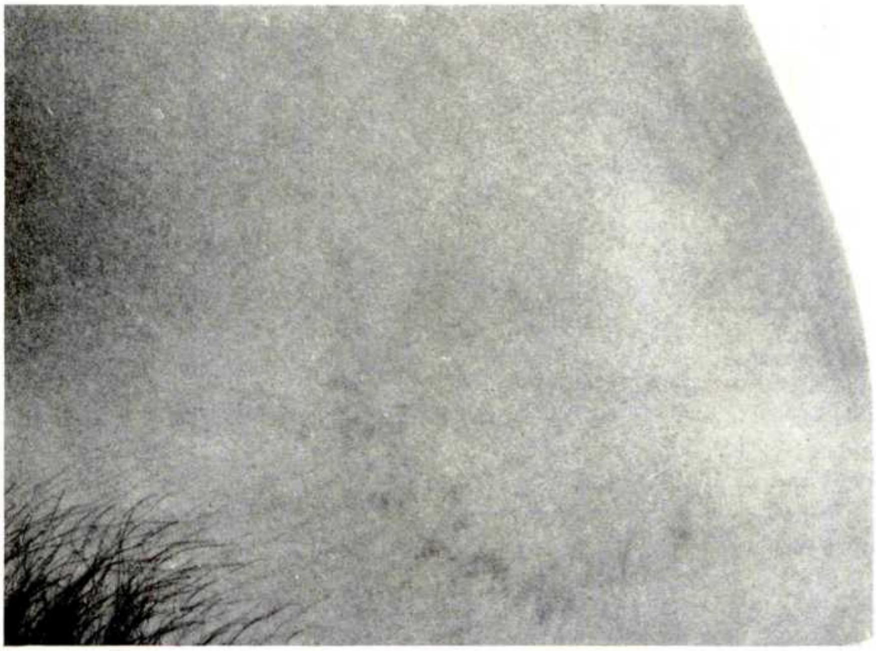

FIGURE 1. Left lower abdomen showing irregular hyperpigmentation assuming an arcuate form.

An initial biopsy from the left thigh showed a sparse upper dermal inflammatory infiltrate with prominent pigmentary incontinence. No amyloid could be demonstrated with the special stains. A second biopsy, from the lower abdomen, also showed pigmentary incontinence but, in addition, a few dermal papillae contained small hyaline bodies (Fig. 2). These hyaline bodies stained only weakly with Congo red and crystal violet. A portion of this biopsy was taken from the paraffin block and re-embedded in epon for electron microscopy. Ultrastructurally, the dermis contained deposits of a fibrillary material with the fibrils measuring from 8 to 10 nm (Fig. 3). These deposits were typical of amyloid.

DISCUSSION

The unusual pattern of the hyperpigmentation and its limitation to the left half of the body suggested that it might be of naevoid origin. However, both the patient and her mother were quite sure that the pigmentation was not present in early life and had been acquired only recently. In this case, the special stains for amyloid did not produce convincing evidence of the presence of amyloid. The staining properties of Congo red and crystal violet are known to vary considerably in formalin-fixed tissue, and this is particularly likely to occur when the deposits are sparse, as in macular amyloidosis (Black, 1972b). Recently, Goodman, Abele & West (1972) have emphasized the usefulness of electron microscopy in the definitive diagnosis of systemic amyloidosis when special stains give inconclusive results. Electron microscopy was also necessary for the specific confirmation of macular amyloidosis in our patient.

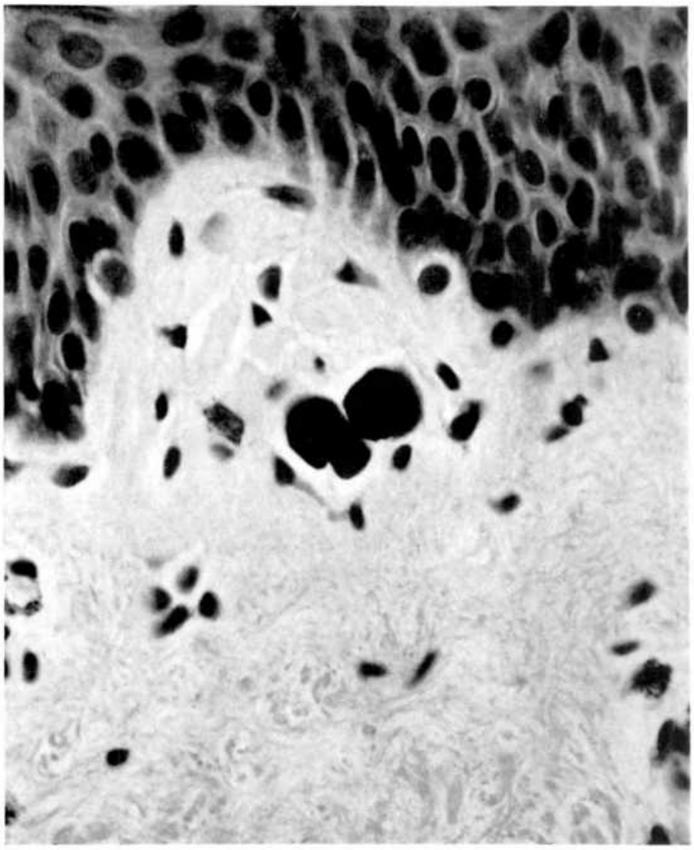

FIGURE 2. Biopsy of macular pigmented area on lower abdomen. Small hyaline bodies are situated in a dermal papilla above some melanin-laden macrophrages (H & E, × 400).

Our experience with this patient indicates that in cases presenting with unusual or bizarre patterns of hyperpigmentation, amyloid should be specifically looked for in biopsy material.

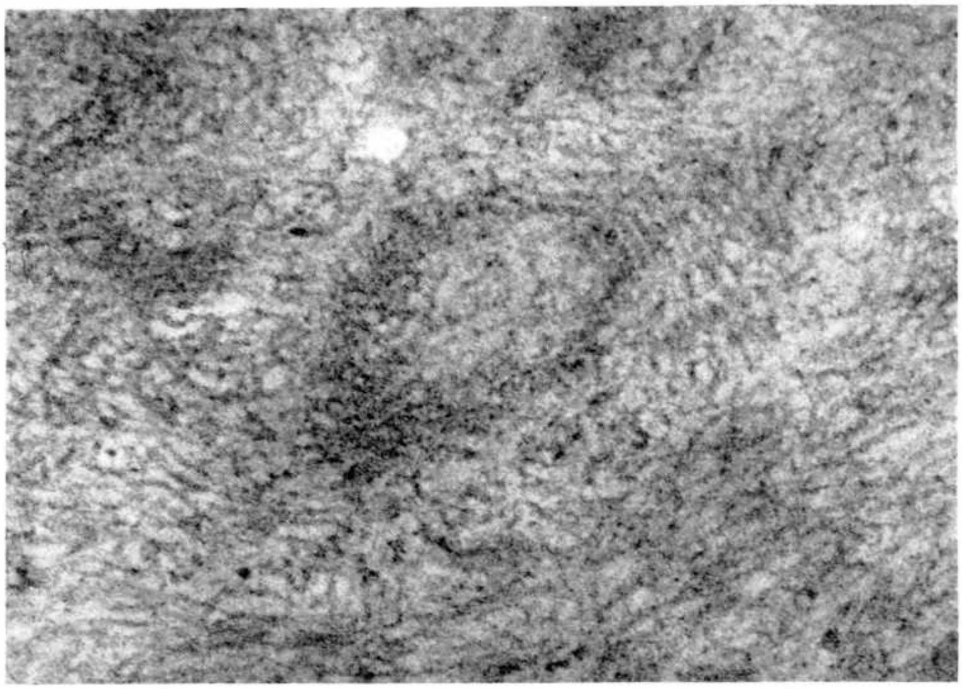

FIGURE 3. Typical amyloid fibrils in the papillary dermis (×95,000).

ACKNOWLEDGMENTS

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Contribution to the knowledge of normolipaemic plane xanthomatosis

G.WEBER AND M.PILGRIM

Skin Clinic, City Hospital, Nürnberg, F.R., Germany

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SUMMARY

The case is reported of a 61-year-old man with a 25-year history of normolipaemic xanthomatosis involving almost the whole integument. He also presented a γ-G-paraproteinaemia, and a bleeding tendency manifested by petechial haemorrhages in the xanthomatous skin, by intestinal haemorrhages and by a subdural haematoma.

In 1962, Altmann & Winkelmann described a previously unknown form of xanthomatosis, characterized by diffuse skin xanthomata, with normal blood fatty acid and cholesterol levels. This form of xanthomatosis is unusual for the diametrically opposed quantitative behaviour of the blood and skin lipids. Our own observation, which is to our knowledge the second in Germany (Müller, 1962), seems to us to be worthy of a detailed report since the clinical picture and course of the disease deviate from those of the known types of xanthomatosis by the development of further complications.

CASE REPORT

Our neurological clinic (Director: Professor Dr F.W.Bronisch) showed us a 61-year-old patient who had been admitted with a tentative diagnosis of cerebral tumour. The neurological diagnosis of a subdural haematoma without preceding trauma was confirmed on trephination.

History

No relevant illnesses are known in the family. In 1919 the patient suffered from infective hepatitis. Since 1945 he has had progressive skin changes. In 1968 he underwent partial gastrectomy for a duodenal ulcer. After admission to the neurological clinic at Nürnberg on 25 July 1970 he developed a massive intestinal haemorrhage of uncertain cause and localization.

On examination

His general condition was somewhat impaired but his nutritional state was satisfactory. The liver was palpable 3 cm below the costal margin. Otherwise he was clinically normal apart from the skin lesions.

Skin condition (Fig. 1)

Nearly the whole skin surface was discoloured giving the impression that the patient had been covered

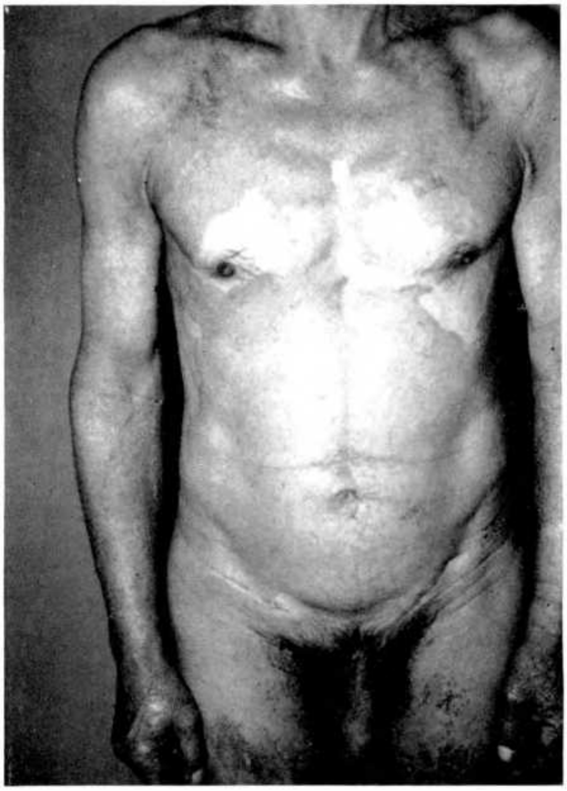

FIGURE 1. Normolipaemic plane xanthomatosis.

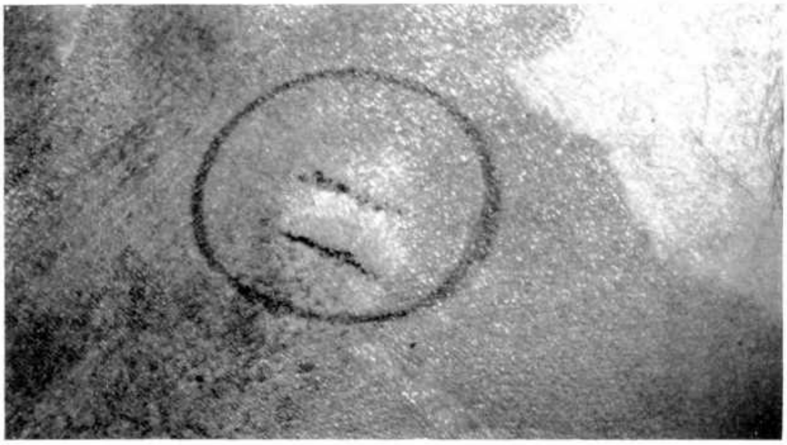

FIGURE 2. Petechiae induced by pinching xanthomatous skin.

with yellow ochre paint. Approximately 1 mm in thickness, the yellow covering showed sharply defined, bizarre map-like ramifications. Around the nipples, areas of skin approximately 10 cm in diameter were unchanged, except for a few small elevated flat-topped yellow plaques. The periphery of these areas consisted of closely aggregated tiny elevated nodules. Similar areas of virtually normal skin were present on the back, above the symphysis and on the lower extremities. The discoloured skin was of velvety soft texture; slight pressure induced petechial haemorrhages (Fig. 2). Extensive spontaneous petechial haemorrhages were present around the thoracic inlet and over the scapulae (Fig. 3). A punctiform and macular dark brown hyperpigmentation covered nearly the whole extensor surfaces and less conspicuously the flexor aspects of the forearms, the thighs and the lower legs. The mucosae were normal. Neither xanthoma palpebrarum nor arcus senilis of the cornea was found.

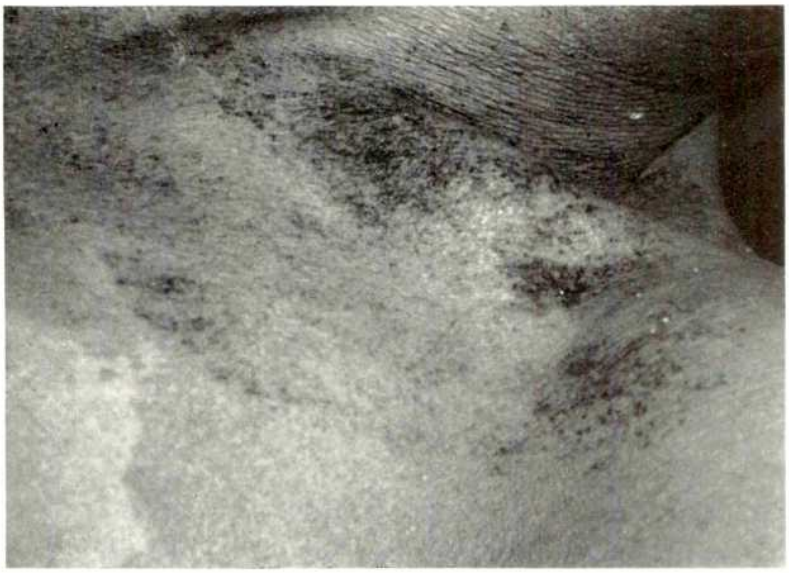

FIGURE 3. Spontaneous petechiae.

Histological findings

A skin biopsy was taken from the left side of the chin and was reported as follows (Professor Dr G. Pliess, Director of the Pathological Institute). The epidermis is normal. The cutis shows an extreme fibroplasia which extends to the subcutis. There are irregular groups of macrophages with a clear, fine, foamy cytoplasm in the upper part of the cutis (Fig. 4). A few lymphocytes are also seen. Staining for fat shows that the macrophages contain abundant sudanophilic material. Only a very few of the macrophages show positive birefringence under polarized light.

Laboratory findings

ESR 102/125 mm; Hb 94%, erythrocytes 5×10^6, leukocytes 11,800, stabs 12, segmental cells 80, lymphocytes 6, monocytes 2, platelets 350,000; Quick's test 100%, bleeding time 1 min 55 s; clotting time 3 min 30 s. Thrombelastogram N.A.D. SGOT 21 MU, SGPT MU, bilirubin 1.4 mg%, alkaline phosphatase 300 mU/ml in several controls. Electrophoresis: protein + α_1-globulin 46·2 rel.%, α_2-globulin 8·82 rel.%, α_3-globulin 4·69 rel.%, β-globulin 9.07 rel.%, γ-globulin 31.13 rel.% with a total protein of 6.1 g%. Bence-Jones-proteinuria was not detected in the urine. In the X-rays of the

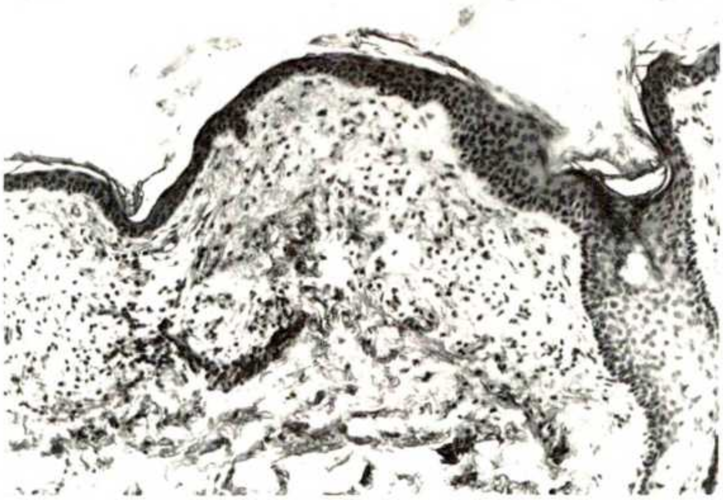

FIGURE 4. Extreme fibroplasia in the cutis, with irregular clusters of macrophages with clear, fine foamy cytoplasm, containing fats (Sudan III).

cranium, cervical spine, lumbar vertebral column, pelvis and thorax there was no evidence of osteolytic metastases of a plasmacytoma. Immuno-electrophoresis (Professor Dr G.Hillmann, Director of Chemical Institute, Nürnberg): with polyvalent and specific antihuman sera there was a deformed the double γ-G-line with enormous antigen excess in the anodic half. The precipitate lines of the other immunoglobulins were diminished. This result indicates a γ-G-paraproteinaemia. The determination of lipids in the serum (Table 1) showed no pathological deviations. In the lipid electrophoresis the pre-β-lipoproteins were reduced, the α-1-lipoproteins somewhat increased.

DISCUSSION

The above report concerns a 61-year-old Caucasoid with plane normolipaemic xanthomatosis with γ-G-paraproteinaemia. This form of xanthomatosis cannot be accommodated in the classification of Frederickson, Levy & Brown (1970) of primary disorders of lipid metabolism. The clinical picture of our patient is marked by generalized xanthomatosis extending over large areas of the skin, over 25 years and associated with a general tendency to bleeding. This resulted in mechanically producible and

TABLE 1. Serum lipids in plane xanthomatosis

Lipid	Standard (mg%)	Patient (mg%)
Total lipids	850	731
Phospholipids	250	220
Total cholesterol	250	194
Cholesterol ester	175	146
Cholesterol + fatty acids	295	243
Free cholesterol	75	48
Triglycerides	230	220

spontaneously occurring petechial haemorrhages (Fig. 2) which were combined with classic haemosiderin pigmentation, most marked in areas of the body exposed to trauma. Haemorrhages in the skin occurred only in areas where there were xanthomata. It seems not unreasonable to consider this tendency to bleed also as a cause of the subdural haematoma and of the massive intestinal haemorrhage. It must be emphasized that the thrombelastogram, Quick's test, the number of platelets and the bleeding and clotting-times showed no pathological abnormalities. Therefore, the tendency to bleed cannot be considered a consequence of a demonstrable defect in the clotting mechanism. One is tempted to assume that the increased tendency to bleed may also explain the development of xanthomata as a result of increased permeability of the vessels for fats. The fact that the vascular fragility is initially limited to the xanthomatous areas may be significant. Further support for this suggestion is the centrifugal extension of the lesions. The histological basis of these changes combines the typical manifestations of xanthomatosis (xanthoma cells which accumulate neutral fats and few lipids) with extreme fibroplasia extending to the subcutis, and also haemosiderin deposits which are not features of xanthomatosis.

Winkelmann & Welborn (1969) considered that xanthoma formation with normal values of serum lipids was a consequence of histiocytic proliferation within the skin; according to Tappeiner & Wodniansky (1970) it is a partial manifestation of a systemic reticulosis. In our case the histological findings did not suggest that the xanthomatosis was a secondary manifestation. A proliferative process in the connective tissue as the primary event with secondary xanthoma formation and subsequent haemorrhage should also be detectable beyond the actual xanthomata. But this fibroplasia is found only towards the centre of the foci, thus stressing its secondary character. Feiwel (1968) explains the induction of xanthomata with paraproteinaemia by an antibody-like influence of immune globulins on cutaneous lymphoreticular cells. Quite obviously, the paraproteinaemia observed in our patient is not an accidental association but part of the process of plane normolipaemic xanthomatosis. Moreover, Lynch & Winkelmann (1966), Feiwel (1968), Lennard-Jones (1960), Neufeld, Morton & Halpenny (1964), as well as Moschella (1970), have demonstrated its association with diseases of the reticuloendothelial system, especially with multiple myeloma.

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Correspondence

UNILATERAL LOCALIZED HYPERHIDROSIS OVER FACE AND SCALP*

SIR, Unilateral idiopathic hyperhidrosis is rare, but recently Cunliffe, Johnson & Williamson (1972) described three cases and reviewed the literature. The following patient has the condition over a larger skin area than is usual, in the distribution of the ophthalmic division of the fifth cranial nerve.

The patient, a woman aged 63, had noticed for nearly 3 years excessive sweating from both the right side of her forehead and the right side of her scalp. The symptom was worse in summer and in hot weather, occurred at any time during the day but not at night, and was related neither to eating nor drinking. She had had a cholecystectomy for gallstones 15 years previously and ever since had at times taken oral propantheline bromide, up to 30 mg daily.

On examination she showed obvious moistness of the hair over the right side of the scalp.

Investigations

(1) In a bright, sun-filled room, sweat droplets were quickly visible over the right side of the nose, forehead and scalp. No corresponding reaction occurred on the left side.
(2) When the patient was stressed (i.e. a skin biopsy was suggested) the reaction mentioned above recurred.
(3) The starch–iodine test, performed on corresponding areas of the forehead, the frontal and parietal regions and above the ear, was strongly positive almost immediately on the right, with a much slower reaction on the left.
(4) A hot cup of tea did not appear to precipitate her excessive sweating.

She has gained some relief from the topical application to the scalp of 5% poldine methysulphate (Nacton) in 70% alcohol.

Broadgreen Hospital,
Thomas Drive,
Liverpool L14 3LB

JULIAN VERBOV

REFERENCE

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PRELIMINARY TRIAL OF DESONIDE, A NEW TOPICAL CORTICOSTEROID

SIR, We have recently undertaken a small study to assess the relative clinical efficacy of a new topical corticosteroid, desonide (Tridesilon) cream. Patients with symmetrical skin disorders affecting the limbs were chosen for study, and were asked to apply the new cream to lesions on one side of the body and betamethasone 17-valerate (Betnovate) cream to the other side. These compounds were prescribed in identical tubes, labelled Right or Left, with instructions that they should be applied two or three times daily according to the severity of the condition. Patients were given detailed guidance on the manner of applying the unguents so as to avoid intermixture on the treated sites. The contents of the tubes were randomly selected, and unknown to the patients or to the physicians until the completion of the study.

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* This patient was shown at the 9 October 1973 Liverpool Meeting of the North of England Dermatological Society

The objective response was assessed weekly (occasionally fortnightly) for a period of 4 weeks, and the results were recorded on a simple score chart. Patient preference was recorded in terms of subjective assessment of response and cosmetic desirability. On subsequent assessments of results, subjective preference and objective assessment were found to coincide closely, but the overall differences between the two compounds studied were minimal. It can be seen that the results when expressed in a table followed a predictable pattern with better response in the eczema group than in psoriasis. None of the treatment preferences is statistically significant, but when all results are pooled the trial comes out overall slightly in favour of betamethasone.

Response level to drug therapy	Treatment preference			
	Tridesilon	Both equal	Betnovate	Sum
Psoriasis				
None	1	6	0	7
Fair	0	0	4	4
Good	0	1	5	6
Sum	1	7	9	17
Eczema				
None	0	0	0	0
Fair	1	6	3	12
Good	5	8	2	14
Sum	6	14	5	26

Obviously, desonide cream can claim no clinical advantage over existing (and cheaper) preparations, but its approximate equipotency with betamethasone 17-valerate does raise an important issue. Since the introduction of the halogenated corticosteroids, it has been widely assumed that the halogenation *per se* is a factor which contributes significantly to their enhanced clinical potency. It would seem, however, that the removal of a fluorine atom from the triamcinolone nucleus does not significantly reduce its activity, and therefore that fluorination cannot really be regarded as an enhancing factor.

This work does not in any way support the hypothesis that a non-halogenated corticosteroid might have given fewer undesirable side effects than a halogenated compound.

ANGUS MACDONALD

Sevenoaks, Kent

LIONEL FRY

St Mary's Hospital,
London, W.2

Book Reviews

Dermatologische Röntgentherapie. O.BRAUN-FALCO and S.LUKACS (1973) Berlin–Heidelberg–New York: Springer-Verlag. Pp.175, illustrated. Price £5.17.

Although superficial radiotherapy for certain non-malignant conditions has been abandoned by some dermatologists in this country, others feel that the reaction against this treatment has gone too far. It is most interesting to read this handbook from the dermatology clinic at Munich and to compare the views of two eminent German dermatologists with current practice in this country.

This soft-backed book covers the subject succinctly. There are excellent introductory chapters on the physical and biological effects of radiation, dosimetry and methods, followed by sections on the treatment of neoplasia of the skin which, in Britain, is usually carried out by departments of radiotherapy. The indications for radiotherapy in benign disorders are in the main similar to those in Britain. It is recommended that not more than a total of 1000 rads should be given to any area of skin in a lifetime, which accords with my own experience and is, in fact, on the safe side.

The authors find radiation helpful for areas of resistant eczema and psoriasis in suitable cases, and also for psoriasis of the nails. They still use it in severe cases of acne over the age of 17. The best results in radiation for keloids are obtained in small lesions of under 6 months' duration. For large keloids they prefer radiation after surgical excision has healed, in association with intralesional steroids. I am surprised that they rarely use Grenz-ray in port-wine stains and, when they do, they use 100–150 rads on 2 consecutive days, and may later repeat this dose. I have found that 800 rads repeated every 4–6 months up to a total of 10,000 rads is both safe and effective in paling the lesions, so reducing the need for covering cream. They rarely recommend using radiotherapy for areas of recurrent herpes simplex and have no opinion on its value in chronic paronychia. In both these conditions radiotherapy seems to be helpful. Finally, radiation protection is covered.

The safety of superficial radiotherapy in the hands of competent dermatologists and radiotherapists has now been established, but its therapeutic value is much more debatable. This book provides no evidence on this point. A bibliography is given but little specific reference is made to it in certain parts of the text. There is a considerable field for controlled therapeutic trials but until further evidence is available it is wise to keep an open mind and retain the facilities. Meanwhile this book can be recommended to all dermatologists who do so, and is suitable for radiotherapists and their staff.

N.R.ROWELL

The Skin and General Medicine. J.N.AGATE (Ed.) (1973) London: Modern Medicine of Great Britain Ltd. Pp. 58, illustrated. Price £4.50.

This is a collection of essays on the dermatological manifestations of some internal disorders, each contributed by an expert in his subject, with a foreword by the editor.

The nucleus of the collection came from a symposium held in 1971 at the Central Middlesex Hospital. The short papers delivered have been moulded into thirteen well written and well documented chapters. In addition to explanatory charts and black and white illustrations, there are more than 110 excellent illustrations in colour.

If there must be any criticism of such a well produced and useful work, it might be regretted that only four pages are devoted to the important and difficult subject of Drug Eruptions; whereas the Skin and Gut is covered in masterly fashion by Sir Francis Avery Jones, and there are also chapters on Ulcerative Colitis and Crohn's Disease which summarize the recent work of H.T.H.Wilson and D.I.McCallum respectively on these subjects.

The chapter on Disorders of Pigmentation seems sadly brief, and one looked in vain for contributions such as the Skin and the Joints, the Skin and the Vascular System, Hirsutism and many others. The organisers of a symposium are limited by time in choosing the subjects to be presented and the authors must inevitably be brief, but if a monograph is to be published it should, perhaps, cover all important aspects of its subject.

Though written perhaps mainly for the general physician interested in dermatological complications, this slim volume will prove useful and helpful to dermatologists and post-graduate students.

P.D.C.KINMONT

Skin Manifestations in Neoplastic Diseases. V.ANDREEV (1973) Sofia, 'Medicina i Fizkultura'. Pp. 338, 102 illustrations.

The reviewer has established that there are in Cambridge a number of people who can read Bulgarian, but none who could review this book for the Journal. Nor indeed would it be appropriate to publish a review of a book written in a language which so few of the Journal's readers can understand. However, the existence of this very comprehensive book on the skin manifestations of neoplastic disease should be brought to the attention of all who are interested in the subject, if only because the extremely long bibliography which follows each chapter is so international in its scope, thanks to the exceptional linguistic abilities of the author, that it will inevitably be of great service to any future workers in this increasingly important field of dermatology.

ARTHUR ROOK

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Review

MICROBIOLOGY I

Experimental infections of man with *staphylococcus aureus*

Knight (1972) could review experimental fungal infection of the skin without once referring to the ethical aspect. With bacterial infections this is not possible. To dip into the horrors of the past, I recommend Dowling (1966) on most infections, and Hill (1943) on the gonococcus. Most of the trouble has been with systemic infections, but experimenters who infect the skin must be on their guard too. Even the mild *Serratia marcescens* (*Chromobacterium prodigiosum*), a favourite biological marker on the skin, with its coloured colonies a great help to the less skilled, shows its teeth if given a chance (Ringrose *et al.*, 1968).

The problems are much worse with *Staphylococcus aureus*. Ethics did not bother the brave Garré (1885) or Bockhart (1887) when they rubbed strong cultures of staphylococci into their own arms. Garré's carbuncle and Bockhart's superficial folliculitis hurt no one but themselves, though Bockhart might have been warned by his previous study—an experimental infection with gonococci of the urethra of a man with dementia paralytica, who had died with renal abcesses 10 days later (Hill, 1943). No wonder that studies turned rapidly to the subject of the survival of organisms on the skin, so well reviewed by Rebell *et al.* (1950), rather than to further attempts to create septic lesions.

The arrival of antibiotics was comforting, and experiments were resumed, but later workers again ran into trouble. Elek & Conen (1957), in their most valuable study of the minimal pus forming dose, gave one volunteer chronic furunculosis. Another formed two large abcesses which, in spite of penicillin therapy, 'progressed to the size of an orange, causing fever and other systemic manifestations'. Foster & Hutt (1960) used three subjects; two developed boils and abcesses which persisted for several weeks after they had been forced to stop the study. Duncan, McBride & Knox (1970), stabbing with a lancet through a mixture of *Staph. aureus* and *Streptococcus pyogenes*, produced six severe abcesses. Despite this, Singh, Marples & Kligman (1971) still felt that 'the record of attempts to induce infections with cultures or lesional material is one of astonishing disappointment'. But in the early part of their own study, three subjects showed 'hot, tender furunculoid nodules around the infection site a few days after removing the dressings. Two of these had lymphangitis, axillary lymphadenopathy, and a modest increase in temperature.' After this, their regime was to give each subject erythromycin or tetracycline systemically when the infected areas were biopsied. Antibiotics were also used for any subject with a fever of over 39·5°C.

The case for doing human experiments is that animal data do not apply to man. The virulence of an organism depends on the species it is attacking; a fact used, for example, to tell the human and bovine strains of tuberculosis apart by their different effects on the guinea-pig and the rabbit. This is clearly true; but the high cost of human work—and Pappworth (1969) has something to say about the use of medical students, mental defectives, and prisoners as 'volunteers'—means that all possible information must first be extracted from relevant animal and *in vitro* studies.

James & Macleod (1961) used, in mice, the methods worked out by Elek & Conen (1957) for humans. Their answers were broadly similar, in that the use of sutures cut down the number of organisms needed to cause sepsis. They found that human hair was much less good than most suture materials at picking up staphylococci from cultures. This is an interesting side-light on the recent epidemic of post-operative wound infections spread by medical staff with staphylococci on their hair (Dineen & Drusin, 1973); but the study of infected human hairs, used as a suture material in mice, seems too artificial to give results useful for man.

The report by Bennett *et al.* (1968) has close animal parallels. They used staphylococcus strain 502A, specially picked as harmless, to colonise the noses of children in an institute for the mentally retarded. The idea was to reduce skin sepsis caused by more unpleasant strains. The children proved difficult to colonise. About 6 weeks later an epidemic of impetigo broke out and, for this, tetracycline was given to many of the patients. Strain 502A, which is relatively resistant to tetracycline, was 'recalled' to the nares of some of those who had apparently ceased to carry it. The faintly spiritualistic term 'recall' had been coined earlier by Simon (1963a), who showed this effect in experimentally infected guinea-pigs. Simon (1963b) also found something rather sinister in the guinea-pig: giving oral tetracycline cut down the dose of tetracycline-resistant organisms needed to produce a standardized skin lesion. Tacking (1954) had already shown that, with rabbits at least, giving penicillin at the same time as a penicillin-resistant staphylococcus greatly increased the death rate. Does it follow that, in man, giving the wrong antibiotic will actually enhance the virulence of staphylococci or lower host resistance? In the study by Bennett *et al.* (1968), 502A, then thought to be harmless but the cause of one recent death (Houck, Nelson & Kay, 1972), was isolated in pure culture from one abcess, and there was an unexpected increase, possibly a 'recall', of tetracycline-resistant staphyloccus type 80/81.

Work with animals raises questions which apply to man, but it cannot answer them. For example, repeated infections of the skin of a rabbit with staphylococci may lead to a state in which abcesses can be caused by inocula too small to affect normal rabbit skin (Johnson, Cluff & Goshi, 1961). If the same happened in man, it might explain why it is so hard to get rid of chronic furunculosis. Certainly in man a recent infection alters reactivity to one major staphyloccal antigen, teichoic acid, as judged by the necrotic reaction to intradermal tests (Martin, Crowder & White, 1967). Against this, one of the volunteers in Elek & Conen's study (1957) was repeatedly infected over a period of 2 years with no obvious change in the number of organisms needed to produce sepsis; and the biphasic reaction of intradermal staphylococcal alpha toxin is different from that in the guinea-pig and rabbit (Smith *et al.*, 1963).

Foster (1971) has shown how difficult it is to skip between species. He took strains of *Staph. aureus* from a variety of human infections and tested them against mice and guinea-pigs. For the mice he used the well-tried method of Noble (1965) to assess virulence. But this proved ineffective with guinea-pigs, and so he used the ability to cause subcutaneous abcesses after intra-cardiac injection. With these methods, there was no correlation between the virulence of a strain for mice and guinea-pigs. Abscess formation, however, may not be a very sensitive measure of pathogenicity; if he took as his guide the weight loss of the guinea-pigs after the infection, then virulence for the two species tallied rather better. Checking back to the original human lesions, he was faced with the task of grading them: is osteomyelitis worse than a suppurating muscle? His final view, like that of Noble (1966), was that pathogenicity for mice correlated better with communicability in man, i.e. the ability of the organism to cause epidemics, than with the severity of the human lesion from which the strain had come.

Elek's (1959) idea was that staphylococci, like other predatory creatures, hunt in packs. The minimal pus forming dose for intradermal infections in man is about five million cocci; a number unlikely to be met under natural conditions. Organisms probably multiply in the hair follicles, out of reach of the defences, until they reach this number and the balance turns in their favour. Strains isolated from boils tend to be active lipase producers when compared with impetigo strains (Williams, 1969). In the pig also, lipase-producing strains are more likely than lipase negative strains to form boil-like lesions (Jessen & Bulow, 1967). Lipases may give them a slight edge in the lipid-rich depths of the follicles even though they survive no better on the surface of the skin (Lacey, Alder & Gillespie, 1970).

Given the chance, on skin scraped repeatedly with a scalpel, and under occlusion, as few as fifteen organisms can cause sepsis (Foster & Hutt, 1960). Singh *et al.* (1971) have devised a useful way of raising staphylococcal lesions in man, using polythene occlusion: the skin has to be pre-treated with alcohol, to eliminate competition from the resident flora, or perhaps to remove antibacterial lipids (Lacey, 1968). The lesions that develop are not like naturally occurring skin infections, as the subcorneal vesicles are free from organisms, though these are massed on the skin surface. Soluble toxic products diffusing through the horny layers were blamed. The best animal parallel is that of Dajani & Wannamaker (1970), who injected organisms into the dermis of hamsters. On biopsy, organisms were crowded in the dermis but very few were seen in the vesicles which formed below the horny layer.

Marples, Rebora & Kligman (1973) have extended their work to show that topical corticosteroids do not hamper antibiotics in dealing with experimental skin infections. The related studies here are those of Raab (1971) and Raab & Windisch (1969), who looked into the *in vitro* interactions of steroid–antibiotic mixtures, showing that these depend both on which antibiotics are used, and on the concentration of the steroid. Steroids stimulate the oxygen consumption of staphylococci at low concentrations, but depress it at higher ones.

As Elek & Conen (1957) have said, experiments on human volunteers are restricted by their very nature. *Staph. aureus* is still untamed, and instead of jumping through its hoop may be vicious and unpredictable. For the clinician, anyway, the knowledge gained the hard way by those who experimentally infect man will mean much more than any amount of laboratory work. More power to their lancets—but not too much.

J.A.SAVIN

Department of Dermatology,
Royal Infirmary,
Edinburgh

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News and Notices

Italian Society of Dermatology

The fifty-third meeting of the Italian Society of Dermatology will be held in Rome from 10 to 12 October 1974. The main themes will be reticuloses and autoimmune diseases of the skin. There will be a round table on recent advances in gonorrhoea. Enquiries may be addressed to Professor P.Nazzaro, Via San Gallicano, 15/a–00153 Rome, Italy.

University of Cambridge School of Clinical Research and Postgraduate Medical Teaching

Medicine and Mycology

A course on common fungal infections will be held at Fitzwilliam College, Cambridge, on 25–27 September 1974. The course is intended for all clinicians and laboratory workers interested in diseases caused by fungi. It should prove especially valuable to dermatologists and clinicians working in the fields of respiratory disease, leukaemia and reticuloses, and organ transplantation, but it is hoped that stomatologists, obstetricians and gynaecologists, paediatricians and all who use immunosuppressive therapy will find much that is relevant for them. For medical microbiologists the course emphasizes the clinical problems and the role of the laboratory in diagnosis and management. It is less concerned with the technical aspects of medical mycology and the identification of fungal species.

The lecturers will include: R.A.Cawson, G.P.Clein, R.R.Davies, D.B.Evans, E.G.V.Evans, Andrew Henderson, B.M.Herbertson, J.R.Hobbs, Rosalinde Hurley, David Kerridge, D.W.R.Mackenzie, M.F.Madelin, R.R.Marples, S.O.B.Roberts, Arthur Rook, M.E.Snell, and D.C.E.Speller.

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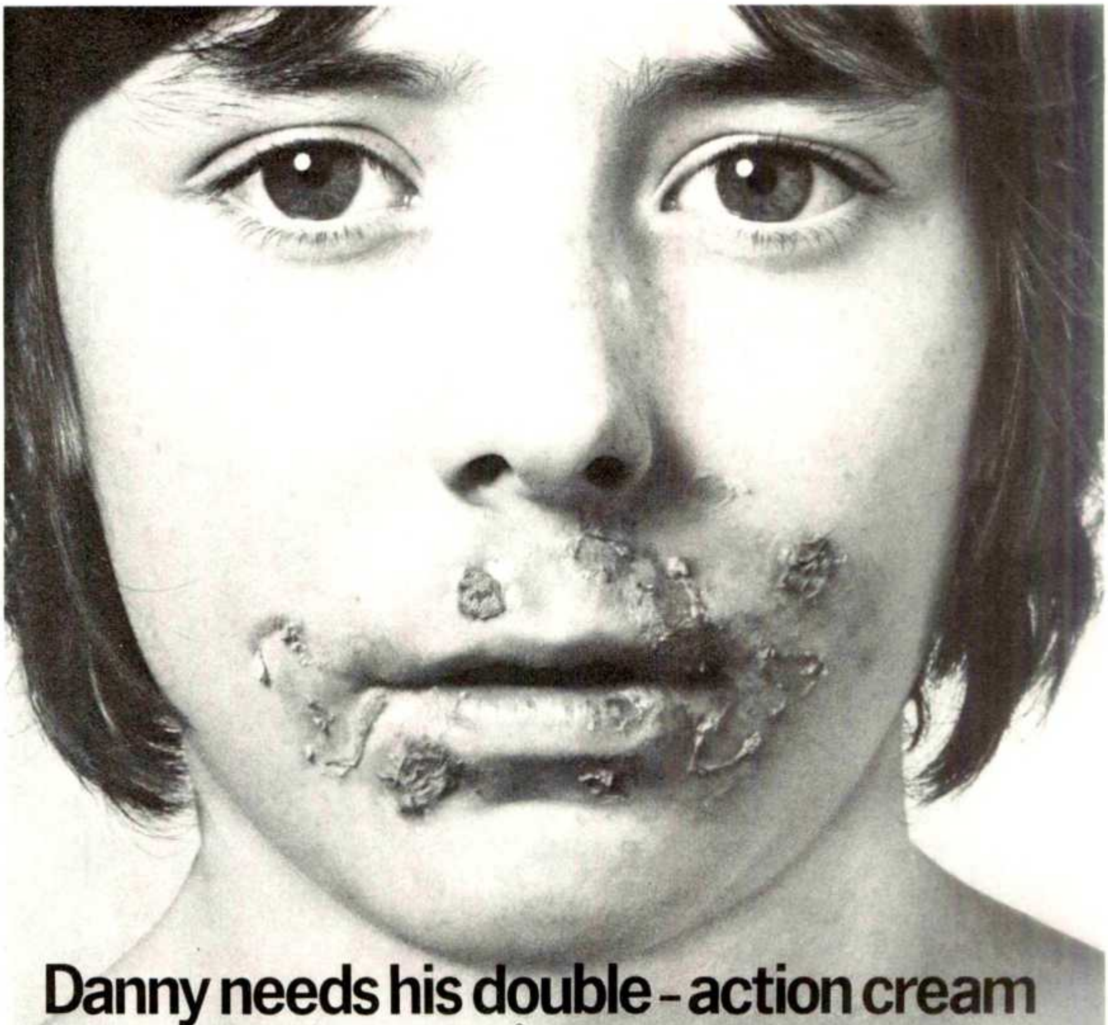

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The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

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John Martin Beare M.D.(Belfast) F.R.C.P. (Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P. (Lond.)
Robert Harold Champion M.A. M.B. B.CHIR. (Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D. (Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S. (Lond.) F.R.C.P.(Lond.)
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Donald William Ross Mackenzie B.SC. PH.D.
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Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
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Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
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Bethel Solomons M.A. M.D. F.R.C.P.I. *Third Edition*, 1973. 268 pages, 107 illustrations (37 colour). Paper, £2.25

Manual of Dermatologic Syndromes
Thomas Butterworth A.B. M.D. MED.SC.M. *Second Edition*, 1972. 104 pages. Lippincott, £1.50

Cutaneous Lesions of the Lower Extremities
M.H. Samitz M.D. M.SC.(Med.) and Alan S. Dana Jr M.D. 1972. 210 pages, 132 colour illustrations. Lippincott, £14.00

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also quarterly reviews of recent advances in contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

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milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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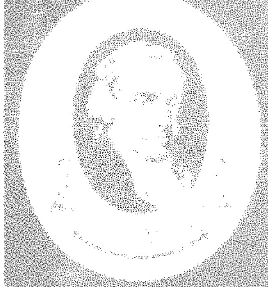

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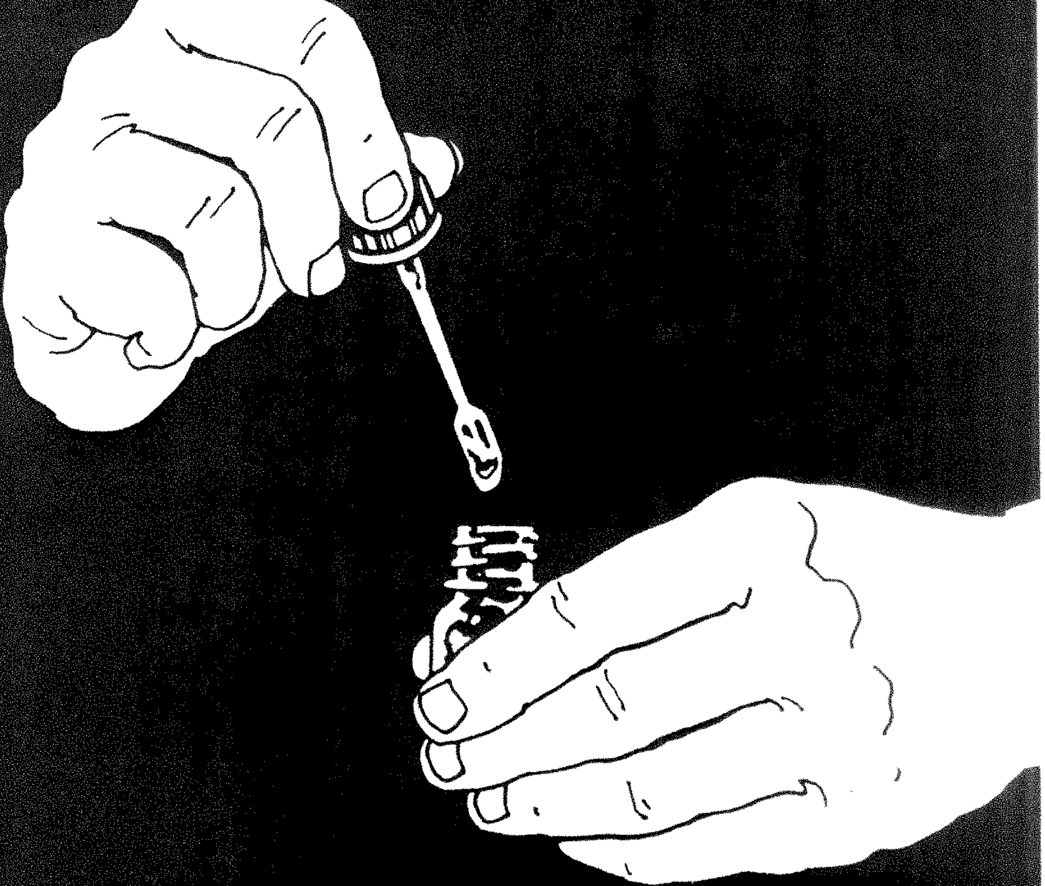

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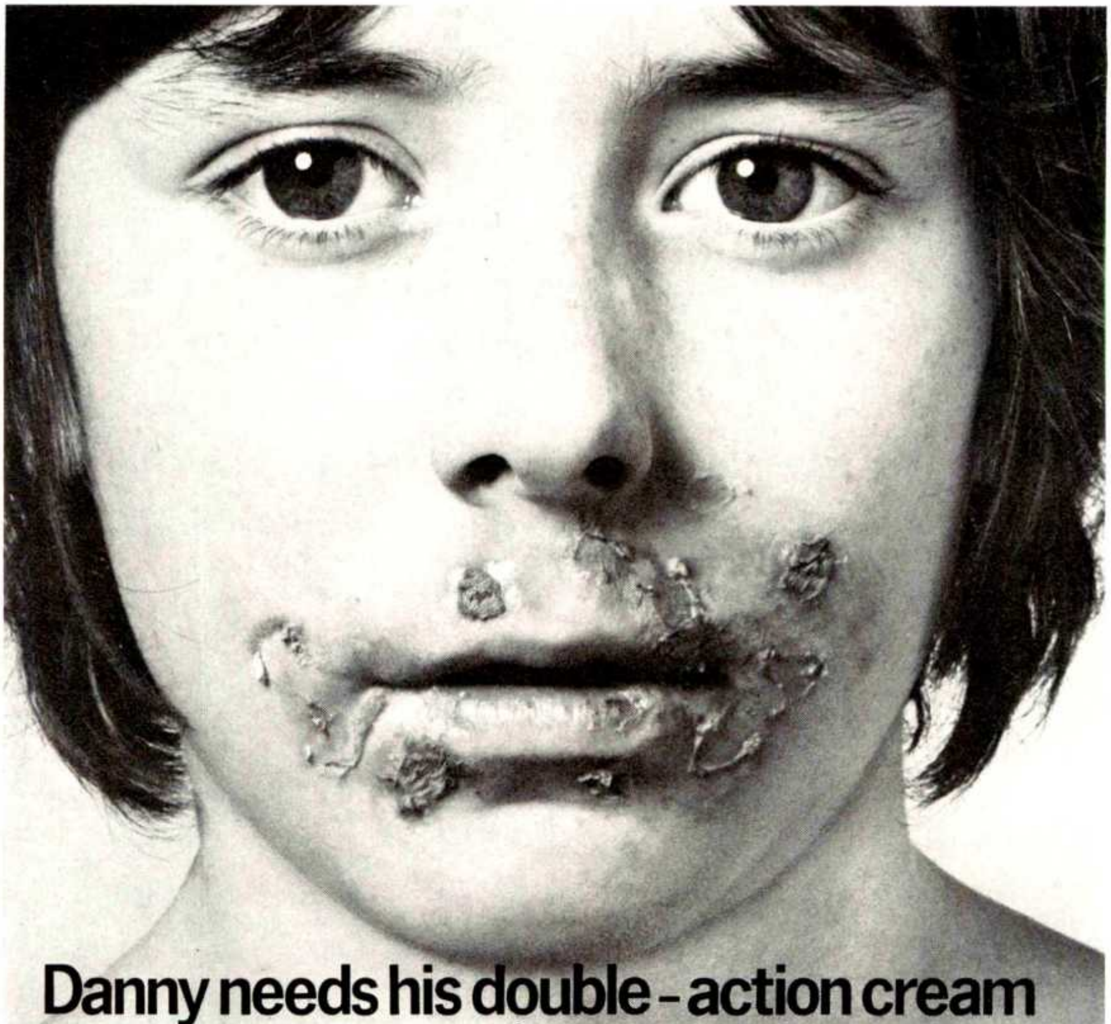

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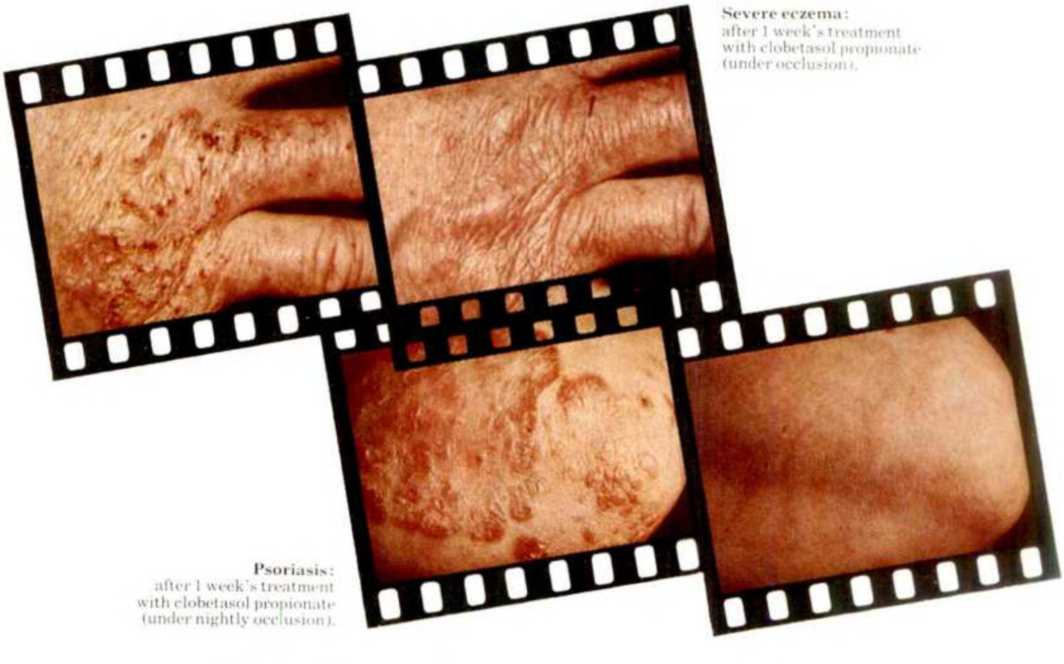

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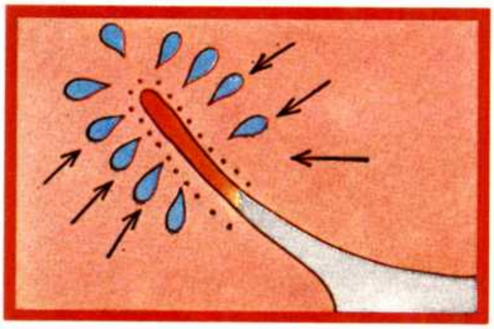

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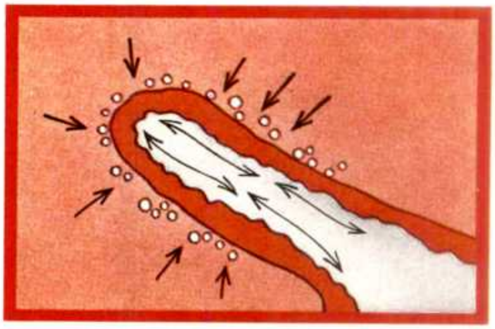

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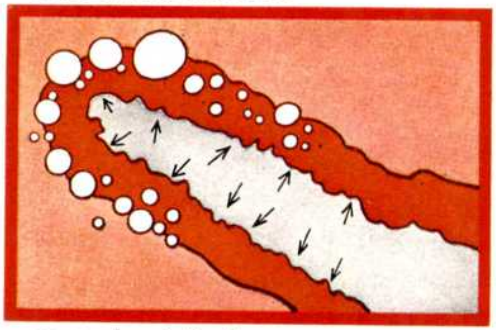

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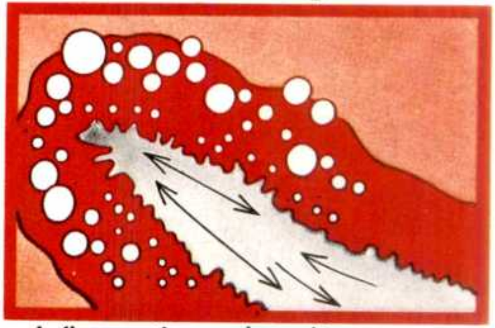

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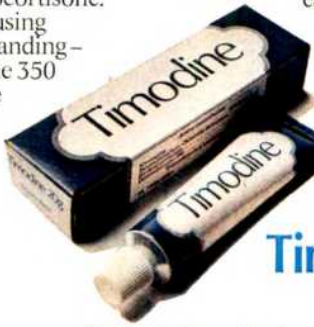

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1 *Indian.J.Dermatol.Venereol.*, **36**, 100, 1970
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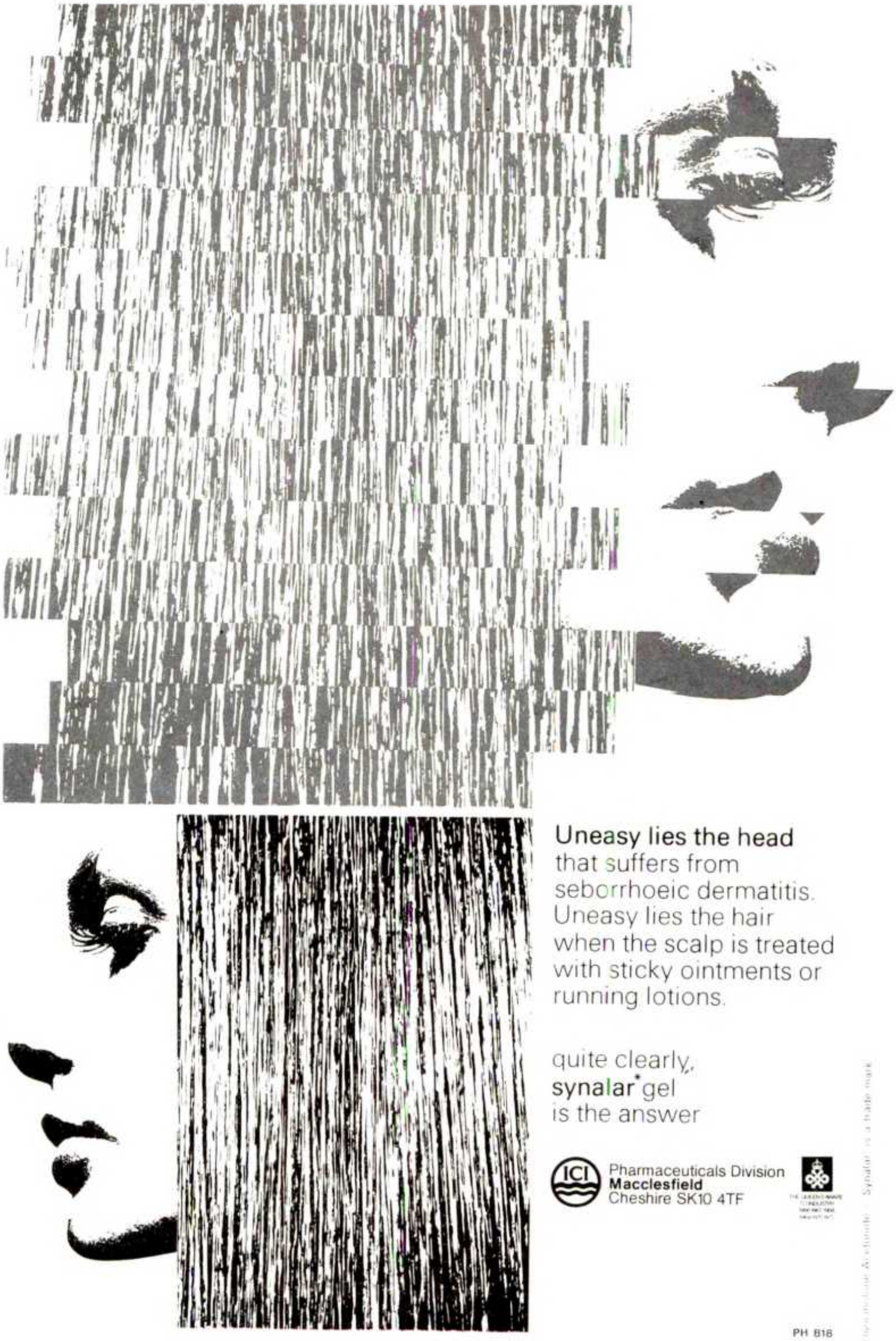

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British Journal of Dermatology (1974) **90**, 481.

Clinical and Laboratory Investigations

Light and electron microscopic studies of physical injury to the skin

I. Suction

J.A.A.HUNTER, EVA McVITTIE AND J.S.COMAISH*

Department of Dermatology, University of Edinburgh, and
* Department of Dermatology, University of Newcastle

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SUMMARY

Suction applied to the skin causes almost immediate paranuclear vacuolization in keratinocytes but does not affect the dendritic cells. If suction is prolonged dermo-epidermal separation occurs at a level between the basal cell membrane and basal lamina.

Stress due to suction of the skin is not commonly encountered in everyday life. Horizontal shear forces are almost certainly those mainly involved in the production of friction damage, but nevertheless both pressure and suction forces are exerted on the foot in ordinary locomotion. Negative pressure may also be exerted on the skin of aviators (especially astronauts) and divers in certain circumstances. Under experimental conditions, suction has been used recently to evaluate dermo-epidermal adherence (Lowe & van der Leun, 1968) and to monitor the state of blistering diseases, such as porphyria cutanea tarda (Copeman, 1970). Some light microscopic and ultrastructural features of suction blisters have already been documented (Kiistala & Mustakallio, 1967; Copeman, 1970), but this paper deals primarily with intra-epidermal changes, which are in sharp contrast to those seen in frictional injury (Hunter, McVittie & Comaish, 1974).

MATERIAL AND METHODS

Twenty-five volunteers were studied, of whom six members of staff had no skin disease and the rest were patients with miscellaneous skin disorders (eczema, psoriasis, dermatitis herpetiformis and porphyria cutanea tarda). Blistering was produced on skin which was macroscopically normal, the flexor aspect of the forearm or the back of the hand being used. The suction chamber chosen was a modification of that described by Kiistala & Mustakallio (1967), and contained a perforated disc with seven apertures each of 5 mm diameter. The negative pressure was usually −150 mmHg but occasion-

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This paper was read in part at the meeting of the Investigative Group of the British Association of Dermatology, 3 February 1973, London.

Reprint requests to Dr J.A.A.Hunter, Department of Dermatology, The Royal Infirmary, Edinburgh.

ally −300 mmHg. Biopsies, with and without local anaesthesia, were taken before and 1, 5, 15 and 25 min after the onset of suction, as well as immediately after the appearance of a blister (70–100 min).

Light microscopy

Part of the biopsy was immediately fixed in formalin for routine histology. Serial sections were stained with haematoxylin and eosin (H & E) or periodic acid-Schiff (PAS). Fresh cryostat sections were prepared for the demonstration of acid phosphatase activity using naphthol AS-TR phosphate and hexazonium pararosanilin according to the method of Barka & Anderson (1962).

Electron microscopy

Cubes of up to 1 mm^3 were immediately fixed in fresh 3% glutaraldehyde in cacodylate buffer pH 7·4 or in a half strength paraformaldehyde–glutaraldehyde fixative pH 7·4 (Karnovsky, 1965). Fixation was either at 4°C or at room temperature for 5–24 h. Some specimens were fixed in 3% glutaraldehyde containing 1% lanthanum nitrate at 4°C for 3 days (Hashimoto, 1970). After thorough buffer washes the specimens were postfixed in buffered 1% osmic acid. Before routine dehydration, most blocks were precontrasted with 0·5% aqueous uranyl acetate (Farquhar & Palade, 1965). The blocks were embedded in Araldite and ultrathin sections were cut with an LKB III Ultramicrotome and contrasted with uranyl acetate and lead citrate. They were examined using an AEI EM6 electron microscope.

Altogether, twenty-five biopsies were processed and ten of these were examined electron microscopically. The results recorded are based on the study of numerous light microscopic serial sections and over 200 electron micrographs.

RESULTS

The light and electron microscopic appearances were similar in all the subjects studied and are best grouped as a whole.

Light microscopy

After less than 1 min of suction, appreciable changes were noted compared with control biopsies obtained without suction. There was slight oedema of the superficial dermis, and paranuclear vacuoles were seen in increasing numbers as the suction time increased; by 25 min nearly all the epidermal cells were affected (Fig. 1), though there was no suggestion of dermo-epidermal separation. Some of the cells extending down follicular walls contained these distinctive vacuoles. They were invariably paranuclear, most often circular or ovoid in cross section, and frequently caused a crescentic deformation of the nuclear outline. The spatial relationship with the nucleus was variable; some vacuoles were superficial and others were deep to or beside the nucleus. Even after 25 min of suction there was no evidence of spongiosis (extracellular oedema). Biopsies taken just after blister formation revealed dermo-epidermal separation, and PAS-stained sections showed that the positive staining basal lamina region remained on the topmost dermis (Fig. 2), forming the blister floor. The cleavage line was frequently clear cut, but sometimes basal cells or dendritic cells (Fig. 2) remained on the floor of the cavity. The roof of the blister was, therefore, formed by the total thickness of the epidermis. It usually, though not invariably, contained numerous vacuolated cells and at this late stage spongiosis could sometimes be seen (Fig. 4). Sections processed to show acid phosphatase activity showed no obvious abnormality in the distribution of the red reaction product.

Electron microscopy

Keratinocytes. The vacuoles in these cells were quite characteristic and were seen in a few cells as early as 3 min after the onset of suction. They were bounded by a membrane (Fig. 3), particularly

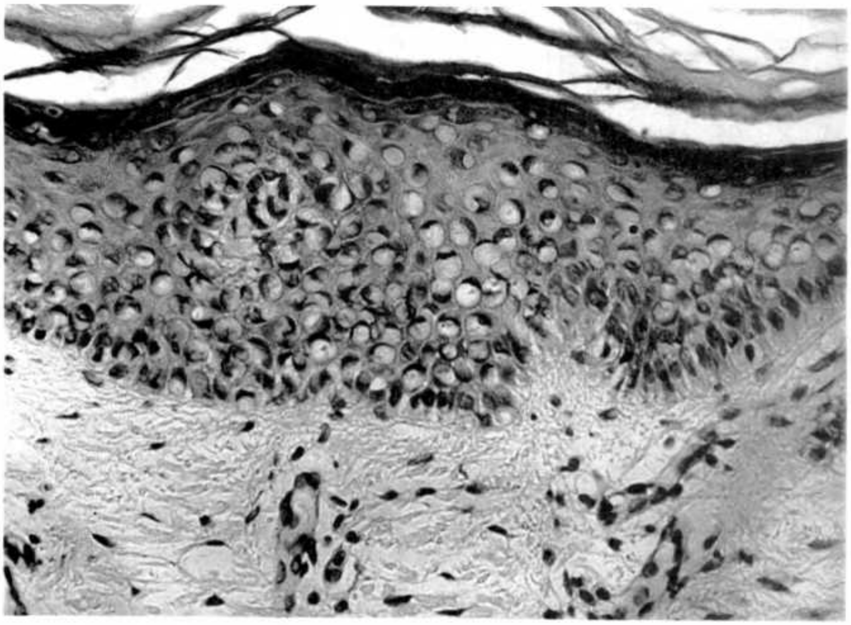

FIGURE 1. 25 min. − 150 mmHg. Nearly all the keratinocytes contain large paranuclear vacuoles, and there is no sign of dermo-epidermal separation (H & E, × 270).

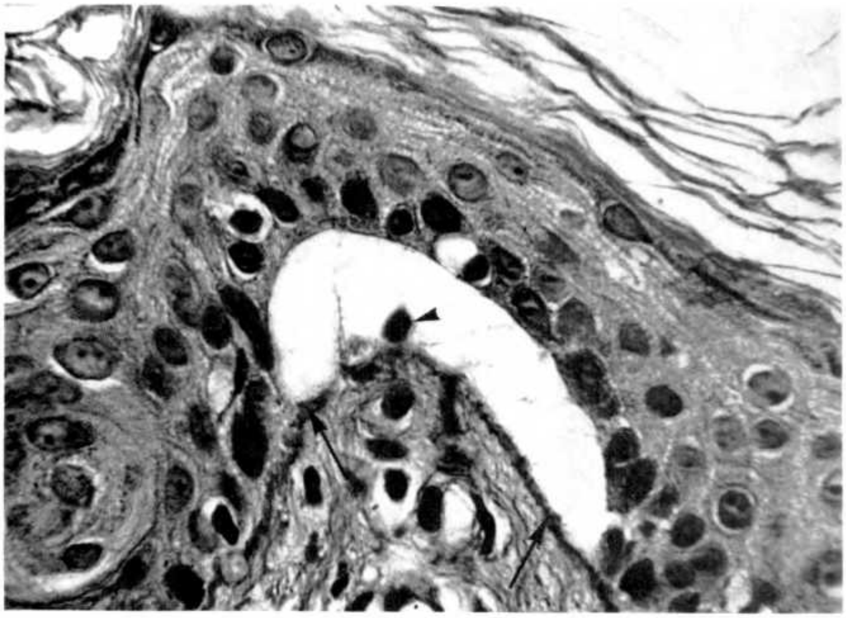

FIGURE 2. 135 min. − 150 mmHg. An early blister forming by dermo-epidermal separation. The PAS positive staining basal lamina region remains on the floor (large arrows). A probable melanocyte (small arrow) has also been left behind on the floor (PAS, × 720).

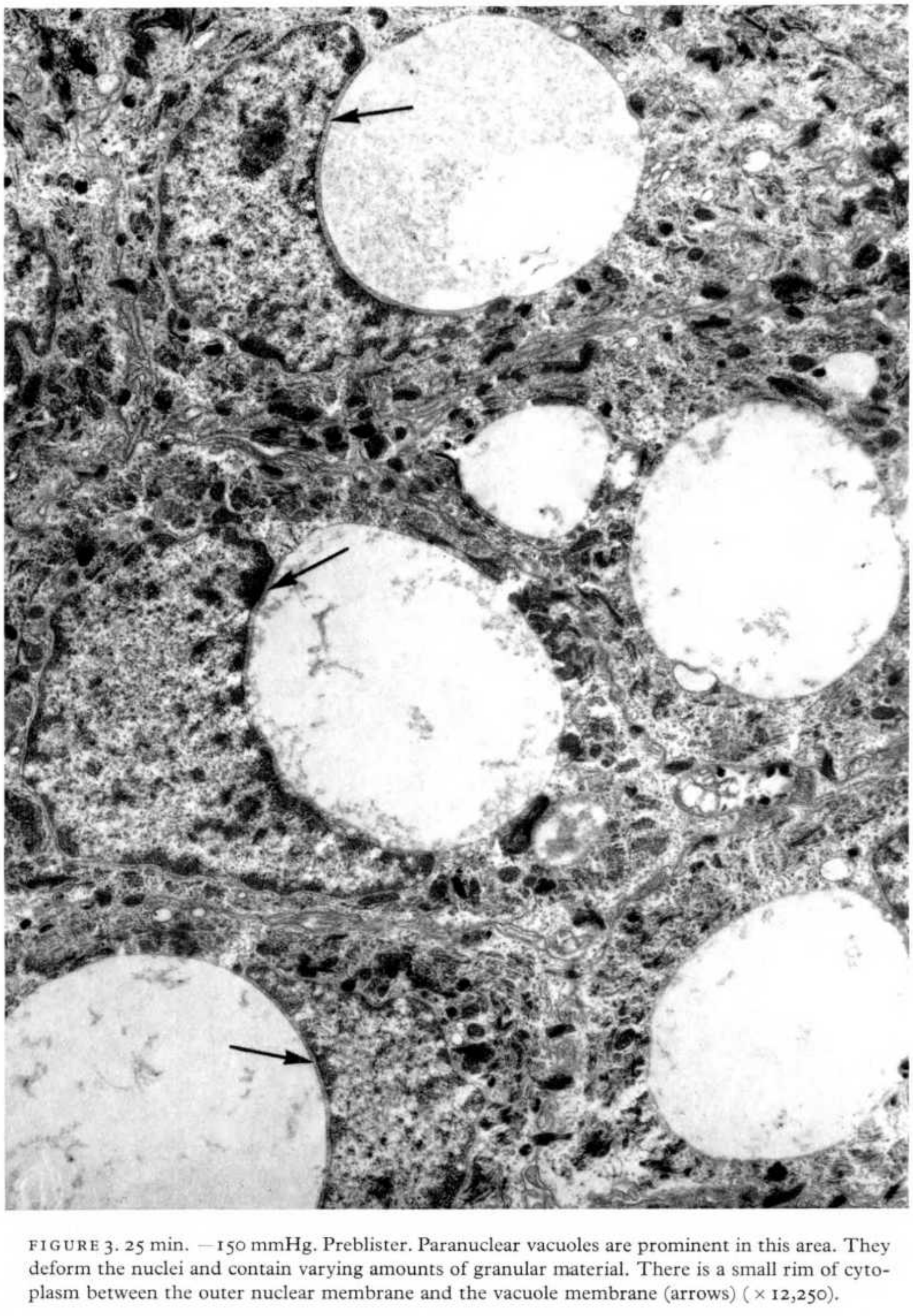

FIGURE 3. 25 min. −150 mmHg. Preblister. Paranuclear vacuoles are prominent in this area. They deform the nuclei and contain varying amounts of granular material. There is a small rim of cytoplasm between the outer nuclear membrane and the vacuole membrane (arrows) (× 12,250).

during the earlier stages of their development, and in some instances this membrane could be resolved into a trilaminar structure. They rapidly achieved a diameter of up to 6 μm, and when they reached this size they were invariably seen next to the nucleus, which they deformed into a half-moon shape (Fig. 3). They were not within the perinuclear space, which appeared to be normal (Figs. 3 and 6). Quite often a thin but distinct rim of cytoplasm could be seen between the outer nuclear membrane and the vacuolar membrane (Fig. 3). They were often filled with a fine granular material that tended to accumulate towards the periphery of the larger vacuoles (Fig. 3). With increasing suction, the vacuoles increased in size and their limiting membranes began to show breaks; their outlines became much less regular (Figs. 4, 6 and 7), and melanosomes (Fig. 8), melanosome complexes (Fig. 7) and membranous remnants (Fig. 6) could be seen within their cavity. At the blistering time, smaller membrane-bound vacuoles (diameter 80–160 nm) could also be seen near the edges of the ruptured paranuclear vacuoles (Figs. 6 and 7). At this late stage, the mitochondia showed degenerative changes. The distribution of the tonofibrils appeared relatively normal, though sometimes they were compressed by the larger vacuoles.

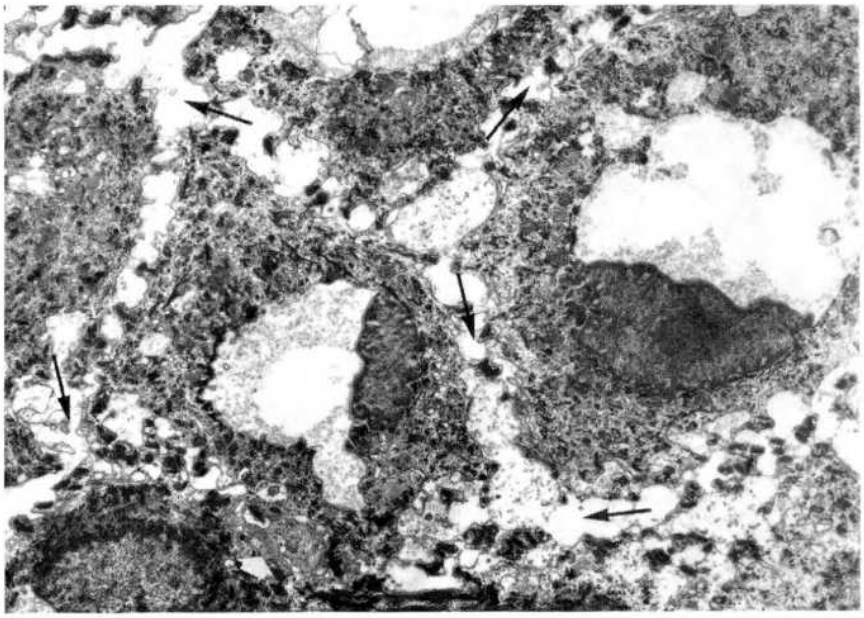

FIGURE 4. 90 min. −150 mmHg. Edge of blister roof. The outline of the perinuclear vacuoles is irregular and there is considerable intercellular oedema (long arrows). The broad white arrow points to a dendritic process of a Langerhans' cell (× 6000).

Dendritic cells. Significantly, neither melanocytes nor Langerhans' cells were seen to contain similar paranuclear vacuoles. Over twenty dendritic cells were seen to be surrounded by keratinocytes containing vacuoles, though they themselves were not affected (Fig. 5).

Intercellular space. This appeared normal at 25 min after the onset of suction, even though most cells contained paranuclear vacuoles. At the blistering time, however, a variable amount of intercellular oedema was seen, particularly at the blister edge, and in some places the distended space contained a fine granular material similar to that in the paranuclear vacuoles (Fig. 4).

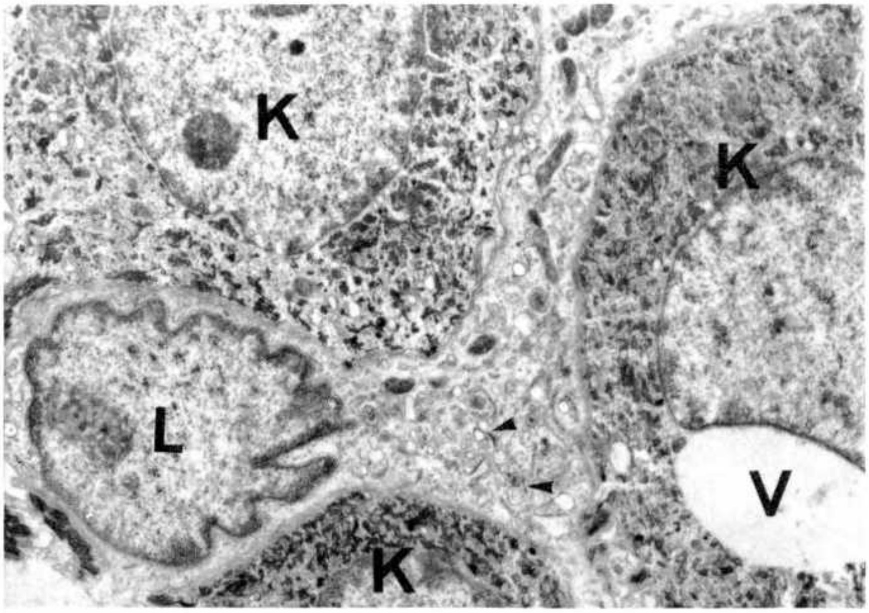

FIGURE 5. 90 min. −150 mmHg. Blister roof. A Langerhans' cell (L) with characteristic granules (small arrows) and dendrites does not contain a paranuclear vacuole. One (V) is seen in a neighbouring keratinocyte (K) (×9600).

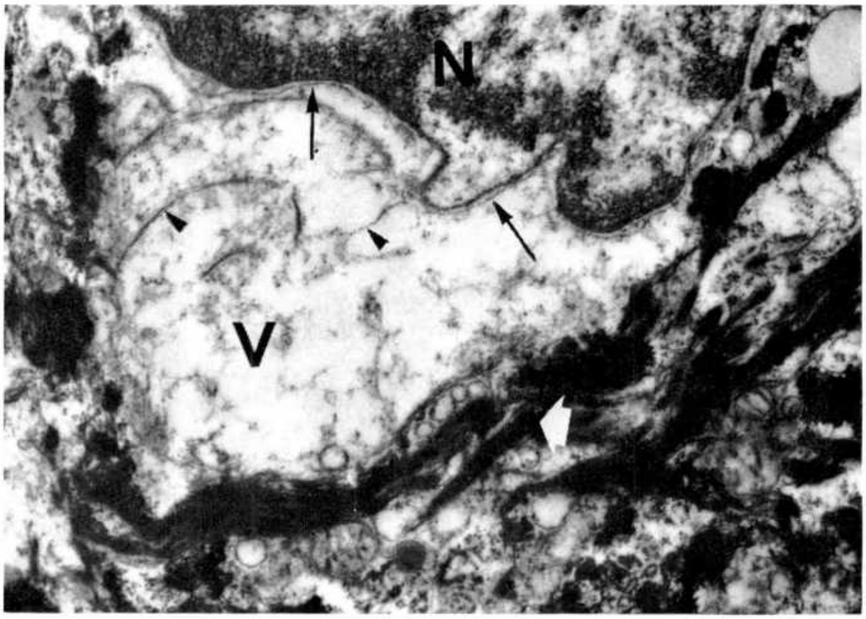

FIGURE 6. 110 min. −150 mmHg. Blister roof. Keratinocyte. The limiting membrane of the paranuclear vacuole contains a granular material and membranous remnants (short arrows). The long arrows point to the intact outer nuclear membrane. N, nucleus; broad white arrow, tonofilaments (×27,900).

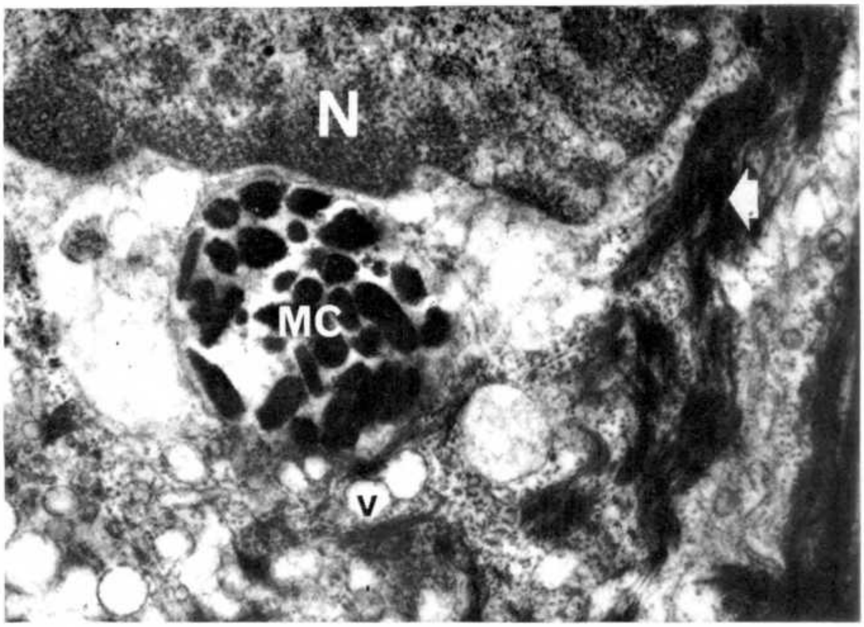

FIGURE 7. 110 min. – 150 mmHg. Blister roof. Keratinocyte. The limiting membrane of the paranuclear vacuole has ruptured and a membrane bound melanosome complex (MC) lies in the remains of the vacuole. Smaller vacuoles (V) are seen at the edge of the disintegrating vacuole. Broad white arrow, tonofilaments (× 37,100).

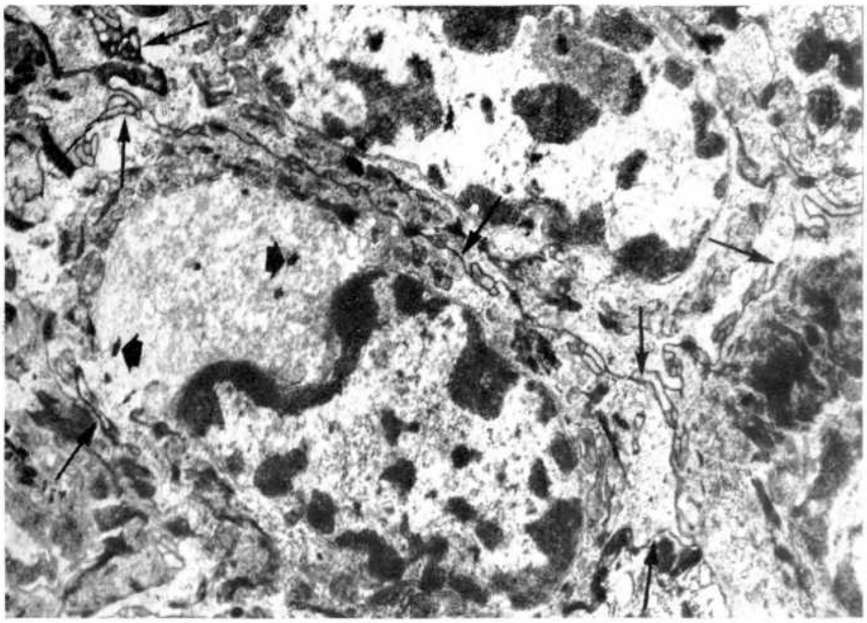

FIGURE 8. 95 min. – 150 mmHg. Blister roof. Lanthanum impregnation. The lanthanum (long arrows) outlines the intercellular space around a keratinocyte containing a paranuclear vacuole, but there is no evidence of tracer within the vacuole. Broad arrows, single melanosomes in vacuole (× 11,400).

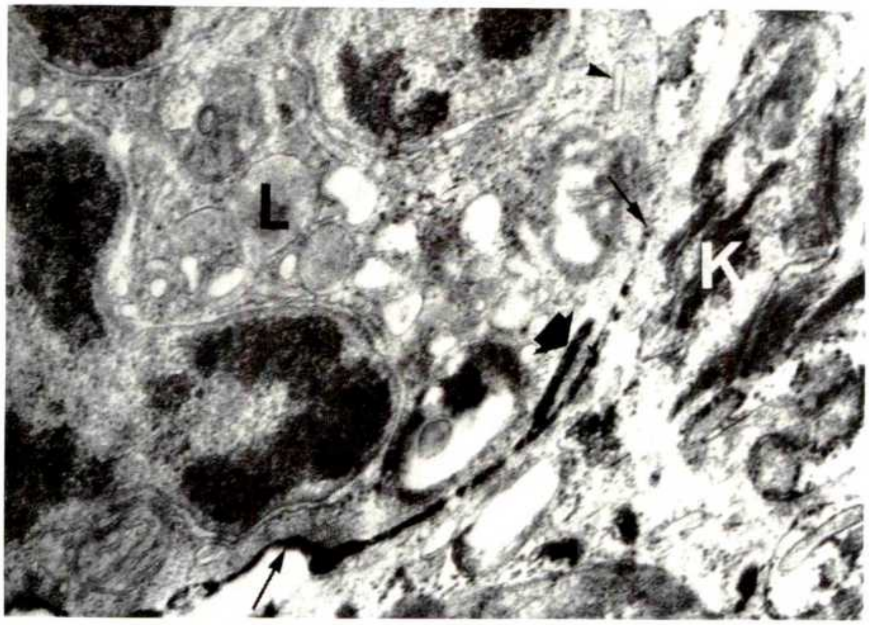

FIGURE 9. 95 min. −150 mmHg. Blister roof. Lanthanum impregnation. The lanthanum (long arrows) stains the intercellular space and a Langerhans' cell granule (broad arrow) attached to the wall of the Langerhans' cell (L). Small arrow points to an unstained granule. K, keratinocyte (× 24,900).

Basal region. The blister was formed by dermo-epidermal separation. Cleavage was most often between the base of the basal cell and the basal lamina, so that the latter usually remained on the floor of the blister. However, fragments of the basal lamina were rarely seen attached to the base of the basal cells in the roof, and sometimes the cleft appeared less neatly through the base of some basal cells. Keratinocytes and melanocytes were occasionally seen in the blister fluid or lying on the floor of the blister.

Lanthanum impregnation. The intercellular space showed a normal appearance when impregnated with lanthanum (Figs. 8 and 9), and the tracer was never seen in the paranuclear vacuoles of keratinocytes (Fig. 8). The lanthanum did, however, stain granules attached to Langerhans' cell walls (Fig. 9).

DISCUSSION

Kiistala & Mustakallio (1967) concentrated on changes at the dermo-epidermal junction when assessing the ultrastructural effects of suction. In this area our findings are in broad agreement with theirs. It is interesting to note the similarity of cold-induced blisters, especially with regard to the site of ultimate blister formation, though in these the epidermal cells are grossly well-preserved (Pearson, 1965). Kiistala & Mustakallio also mentioned that a few keratinocytes contained vacuoles which caused a peculiar crescentic displacement of an otherwise normal-looking nucleus and considered that the vacuoles were situated between the inner and outer nuclear membranes. We would agree with Copeman (1970), who commented that the vacuoles and nuclear displacement were a most noticeable feature of suction and could be seen constantly.

The nature and mechanism of formation of the paranuclear vacuoles present interesting problems. Similar vacuoles have been noted in human keratinocytes in dermographic weals (Cauna, Macy & Cralley, 1970), and have been produced experimentally by ultraviolet irradiation (Nix, 1967), by intradermal injection of hypertonic solutions of sodium chloride and of dextrose (Hönigsmann & Wolff, 1973), by suction (Copeman, 1970), and by the topical application of vitamin A to the human skin (Plewig, Wolff & Braun-Falco, 1971). Wolff & Hönigsmann (1973) believed that the vacuoles seen in this study are identical to those produced by the intracutaneous injection of hypertonic solutions into guinea-pig skin. They have carried out extensive studies to determine their nature and mechanism of formation. Using tracers such as colloidal silver and horse-radish peroxidase in the injected solution, they were able to show staining of both the intercellular space and the paranuclear vacuoles. The vacuoles were stained also with osmium soaking (a technique which outlines the endoplasmic reticulum) and did not contain ruthenium red or nucleoside triphosphatase activity, suggesting that they were not formed by infolding of the cell membrane. They concluded that there was a canalicular system of interconnected cisternal channels within keratinocytes, which formed a direct communication between the extracellular and the perinuclear spaces, and that it could unfold under certain experimental and pathological conditions (Hönigsmann & Wolff, 1973). In this study, the development of the vacuoles was too quick for it to be possible to consider endocytic mechanisms for the uptake of the fluid. The rapidity of their formation suggests also that fluids are sucked from the intercellular space into pre-existing cavities along communications of endoplasmic reticulum, as suggested by Hönigsmann & Wolff.

The precise role of lanthanum in staining the cell surface is uncertain. Doggenweiler & Frenk (1965) considered that it stained lipids of the cell surface, while Behnke (1968) and Shea (1971) felt that its staining was specific for the demonstration of acid mucosubstances. Its delineation of the extracellular space seems to be due to non-specific precipitation (Revel & Karnovsky, 1967). It has been used to outline the extracellular space in human epidermis (Wolff & Schreiner, 1968), and to show continuity between the extracellular space and granules attached to the walls of Langerhans' cells (Hashimoto, 1970). In this study, it was at first surprising to note that there was no sign of lanthanum penetration into the paranuclear vacuoles within keratinocytes. The most likely explanation of this would seem to be that a communication, like that envisaged by Hönigsmann & Wolff, is patent only during the period of suction. As this communication cannot be penetrated by lanthanum in normal skin (Wolff & Schreiner, 1968), Hönigsmann & Wolff have tentatively suggested the presence of a valve or sluice-like mechanism which opens only under certain circumstances, e.g. when in contact with hypertonic solutions or an increased flow of intercellular fluid. If this were so, then the lanthanum, which was used only during fixation of the biopsy specimen, would not be expected to gain access to the postulated canalicular system of communication.

Although ultrathin serial sections were not made, the fact that paranuclear vacuoles were not seen in melanocytes or Langerhans' cells seemed significant. Examination of numerous sections revealed no affected dendritic cells, even though neighbouring keratinocytes contained large paranuclear vacuoles. It should also be pointed out that neither Cauna *et al.* (1970) nor Hönigsmann & Wolff (1973) mentioned such vacuoles in epidermal dendritic cells, in spite of their obvious presence in keratinocytes. There are two likely explanations. First, it is possible that communications between the extracellular space and the system of endoplasmic reticulum, demonstrated in keratinocytes (Hönigsmann & Wolff, 1973), do not exist in dendritic cells. Secondly, it is conceivable that keratinocytes, because of their intercellular connections (desmosomes, intertwining villous processes and intercellular cement substance), are more prone to the effects of suction than dendritic cells, which merely move as a whole in the direction of the suction force.

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Light and electron microscopic studies of physical injury to the skin

II. Friction

J.A.A.HUNTER, EVA McVITTIE AND J.S.COMAISH*

Department of Dermatology, University of Edinburgh, and
* Department of Dermatology, University of Newcastle

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SUMMARY

Frictional injury to the skin causes inter- and intra-cellular oedema of the epidermis, and if severe produces membranous rupture and formation of an intra-epidermal blister. The appearances are consistent with direct physical damage to the cells.

Both for gripping and for moving, man cannot function without friction between him and his environment. However, if this stress is prolonged or unusually severe, pathological changes occur, culminating in blisters and erosions. Human epidermis subjected to frictional stress has been studied light microscopically (Naylor, 1955; Fukuyama & Cortese, 1968), and autoradiographically (Epstein, Fukuyama & Cortese, 1969), but to date no electron microscopic studies have been published. This paper correlates the light microscopic changes with electron microscopic appearances and contrasts both with those produced by suction (Hunter, McVittie & Comaish, 1974).

MATERIAL AND METHODS

Six volunteers with ages ranging from 20 to 78 years were studied. Four had miscellaneous skin diseases (psoriasis, three, and tinea pedis, one). Macroscopically normal skin on the dorsal surface of the forearm was chosen for the experiments. The apparatus used was similar to that described by Naylor (1955), and consisted of a machine which rubbed skin with a reciprocating action at measurable speeds and forces until a blister or its immediate consequence, an erosion, was produced. The head was hemispherical and made of stainless steel. A description of the machine and its operation has already been published (Comaish, 1973). The friction force varied from 1·9 to 3·9 Newtons† and blistering occurred at times ranging from 5 to 14 min. Two of the subjects agreed to having

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Reprint requests to: Dr J.A.A.Hunter, Department of Dermatology, The Royal Infirmary, Edinburgh.

† 1 Newton (N) causes an acceleration of unity (1 metre per second per second) to a 1 kilogram mass.

biopsies taken from four different sites, allowing sequential changes to be studied up to the time of an erosion (e.g. biopsies at 1, 5, 10 and 13 min after the onset of friction).

The light and electron microscopic techniques were as reported in our study on suction blisters (Hunter *et al.*, 1974).

Altogether fourteen biopsies were taken. Ten were prepared for light microscopy and were serial sectioned, and the remaining four (two taken just after blister formation and two approximately midway between the onset of friction and expected blister time) were processed for electron microscopy.

RESULTS

There was no significant difference noted between different subjects, and the results are therefore grouped as a whole. They are most conveniently summarized by describing changes seen at the time of blistering (e.g. 14 min) and those seen much earlier (e.g. 5 min), where the biopsy was taken from intact but slightly erythematous skin. The changes in the latter (hereafter described as 'early' changes) were more subtle and could often be traced only by searching through numerous serial sections with light microscopy, and by carefully checking thick sections embedded in Araldite before cutting ultrathin sections for electron microscopy.

Light microscopy

Early changes. The earliest changes were noted in the upper Malpighian layer (Fig. 1), whilst the overlying horny and granular cell layers were relatively unaffected. The changes were quite frequently noted adjacent to hair follicles and sweat duct orifices, and consisted of a localized area of pale and washed out eosinophilic change in the haematoxylin and eosin sections (arrow in Fig. 1). The cells showed marked intracellular oedema, their margins were indistinct, and there was some spongiosis. Small vesicles could be seen when the changes were more developed (Fig. 1). The superficial dermis showed only mild oedema.

Late changes. Blistering occurred intra-epidermally at the level of the upper Malpighian layer (Fig. 2). Many of the neighbouring epidermal cells in the floor and to the side of the blister showed similar changes to those seen in the early stages and described above. These sometimes extended to the level of the basal cells (broad arrow, Fig. 2), but the dermo-epidermal junction remained intact. However, below the blister the basal lamina region stained less intensely with PAS (Fig. 2). The superficial dermis still showed only mild oedema.

Electron microscopy

Early changes (Figs. 3, 4, 7 and 8). Figs. 3, 4 and 7 are representative of the early changes seen in areas such as that arrowed in Fig. 1. There was obvious intracellular oedema (broad arrows, Fig. 3), which was most noticeable at the cell periphery, and when severe produced a granular appearance in an area devoid of organelles (Fig. 4). As a result of this, the tonofilaments sometimes appeared clumped, resembling necklaces around the nuclei (Fig. 3). In places, small vacuoles (approximately 0·8 μm in diameter) appeared in the oedematous periphery of keratinocytes, and the membranes of these cells were often ruptured (V_1, Fig. 3), allowing their granular contents to spill into small dilatations of the extracellular space (V_2 and V_3, Fig. 3). The few dendritic cells seen appeared to be relatively unaffected.

Late changes (Figs. 5 and 6). Similar though more pronounced changes were seen around established blisters. Figs. 5 and 6 are representative of changes seen in an area adjacent to the broad arrow

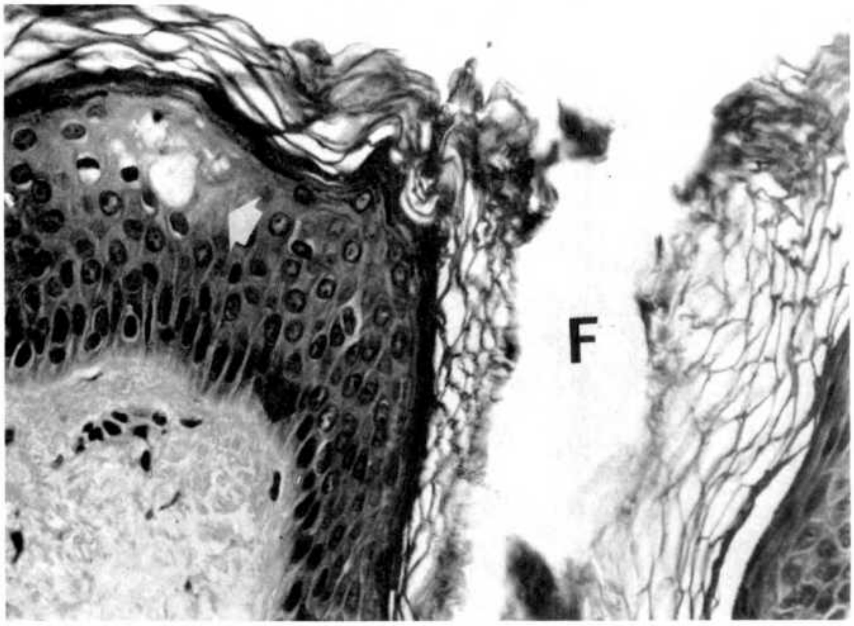

FIGURE 1. Early changes (5 min rubbing, 2·6 N) are indicated by the broad arrow and are seen just deep to the granular cell layer. F, follicle (H & E × 420).

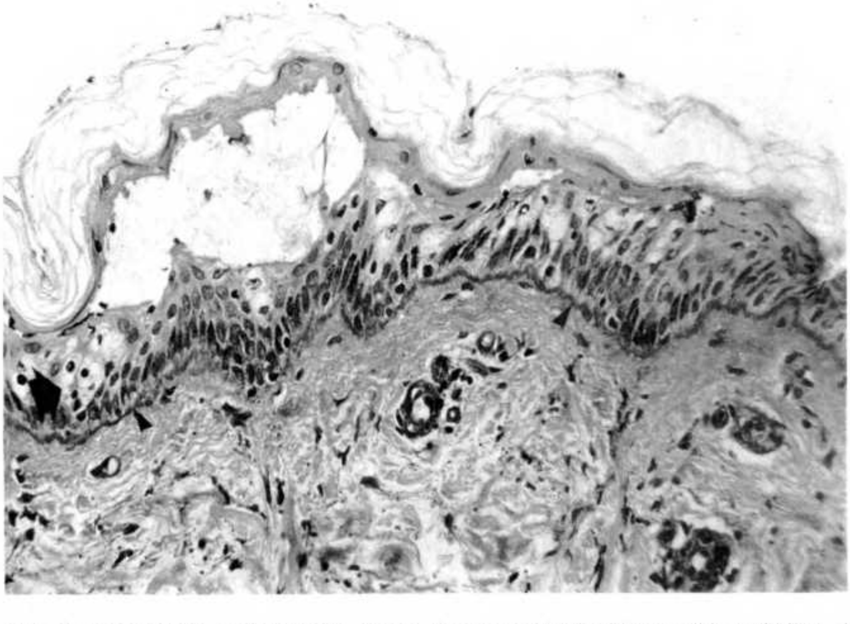

FIGURE 2. Late changes (14 min rubbing, 2·0 N) show an established intra-epidermal blister in the upper Malpighian layer. Broad arrow points to cells in base which show marked intracellular oedema. The basal lamina (small arrows) shows some lack of definition below the blister but is otherwise intact (PAS, × 270).

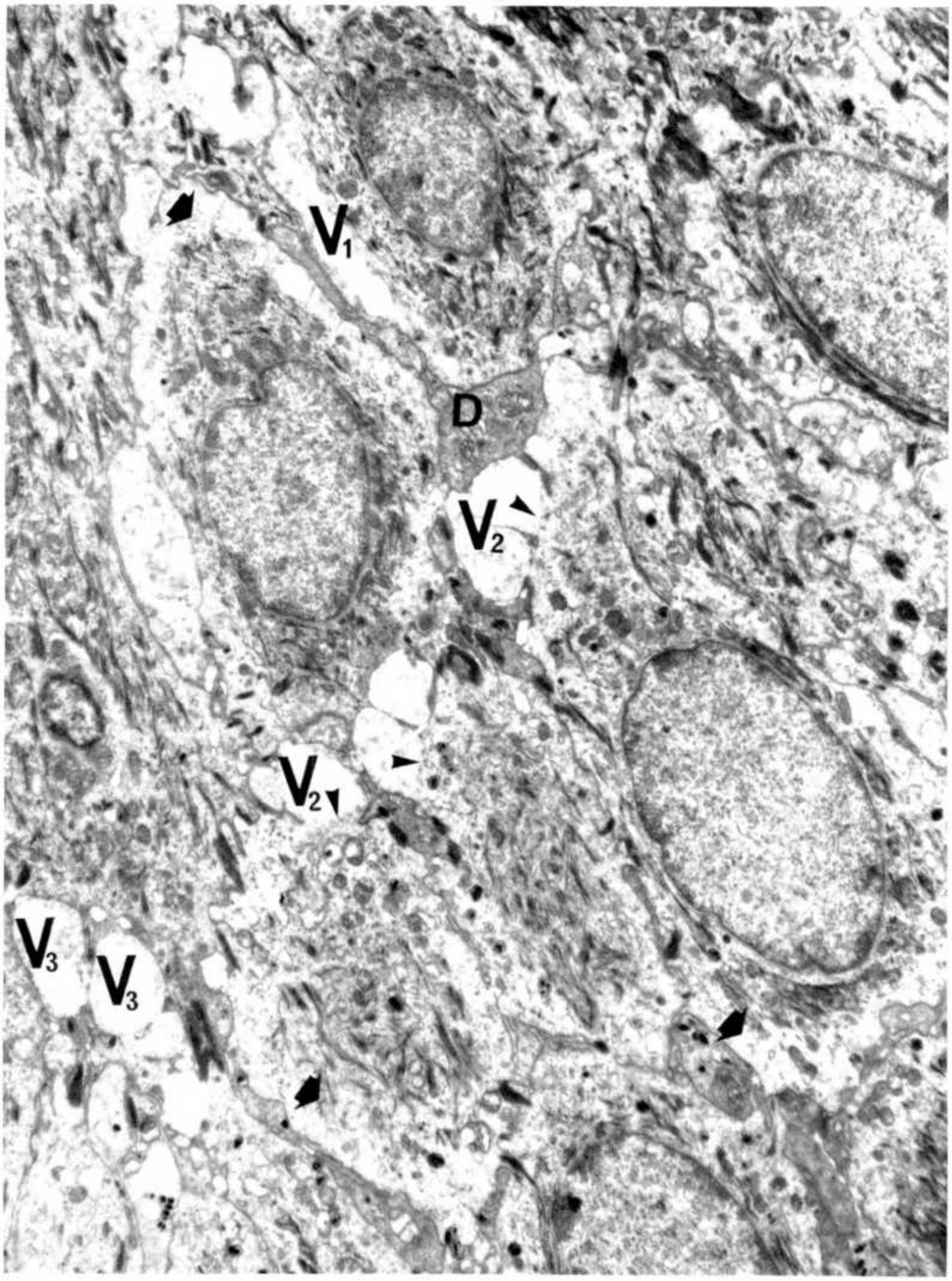

FIGURE 3. Early changes (5 min rubbing) seen in an area similar to that arrowed in Fig. 1. The keratinocytes show marked intracellular oedema (broad arrows) and the cell peripheries are relatively devoid of organelles. Small arrows point to places where the membranes are ruptured. V_1, membrane bound vacuoles in the periphery of the cell; V_2, vacuoles in extra-cellular space containing granular material adjacent to ruptured membranes; V_3, extra-cellular space dilatations; D, dendritic cell (× 7000).

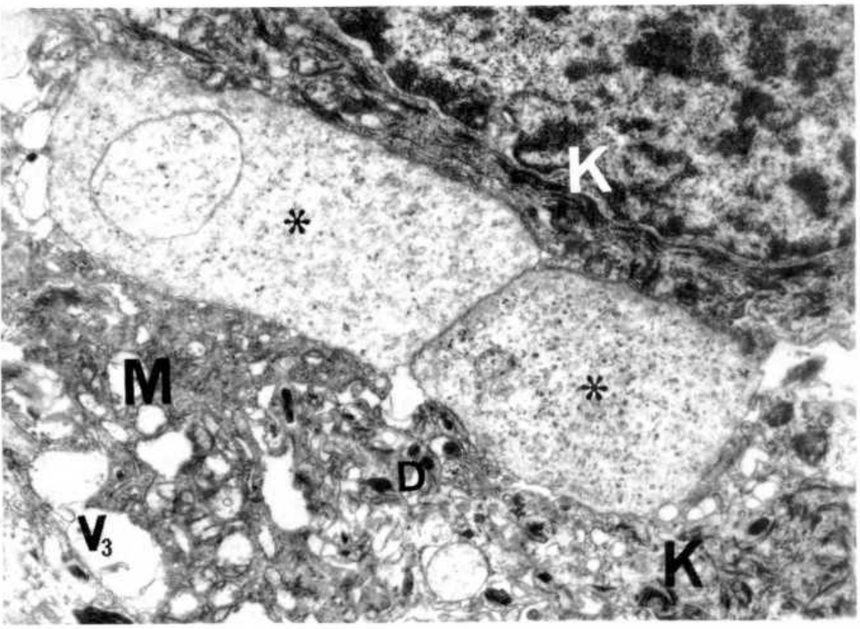

FIGURE 4. Early changes (5 min rubbing). Degenerative remnants of cells (*) contain a granular material and are free of organelles. K, keratinocyte; M, melanocyte; D, desmosome; V_3, extracellular space dilatation (× 11,700).

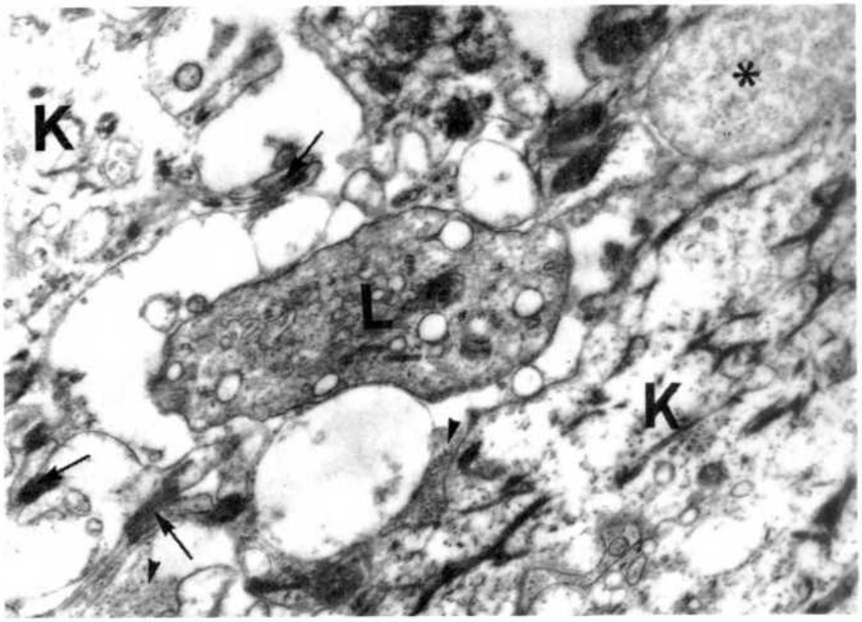

FIGURE 5. Late changes (6·5 min rubbing) at the edge of an erosion seen in an area similar to that indicated in Fig. 2. There are numerous dilatations of the extracellular space and some contain a granular material (small arrows) similar to that seen in degenerative cells (*) and oedematous keratinocytes (K). The process of a Langerhans' cell (L) appears unaffected. Large arrows point to intact desmosomes (× 20,700).

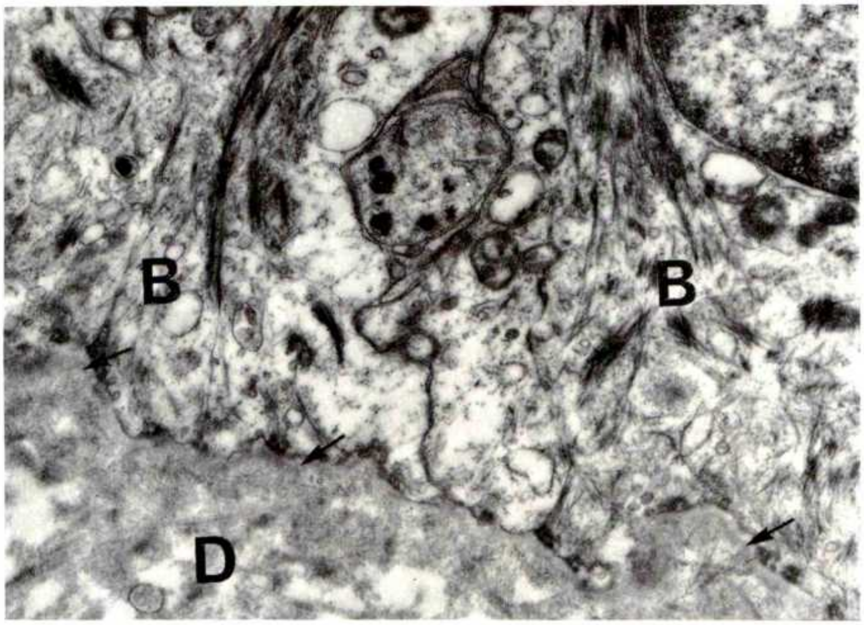

FIGURE 6. Late changes (6·5 min rubbing) seen in basal cells (B) in the floor of an erosion. The basal cells show marked oedema. The basal lamina (arrows), although indistinct in places, is essentially intact. D, dermis (× 17,700).

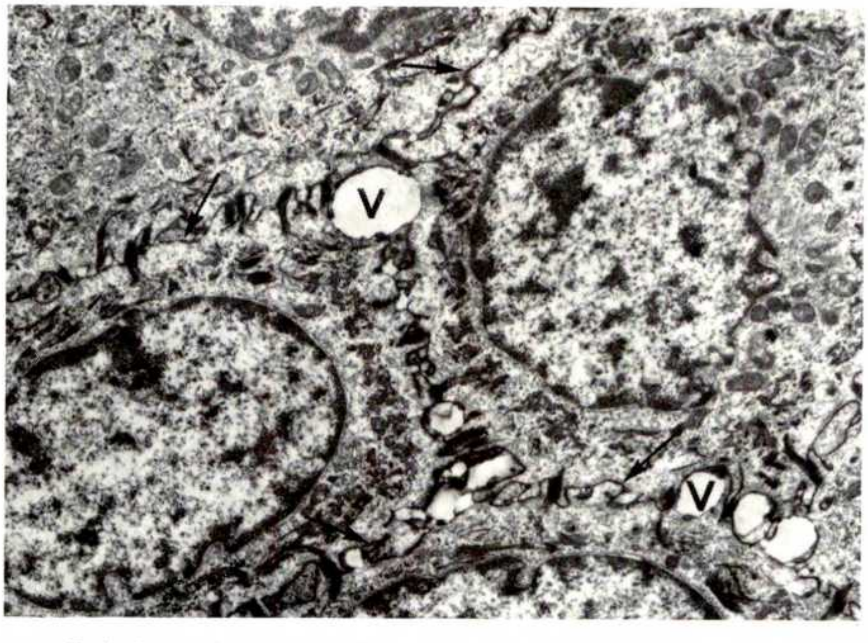

FIGURE 7. Early changes (5 min rubbing). Lanthanum preparation. Lanthanum (arrows) outlines the extracellular space (arrows) and the localized dilatations (V) (× 7200).

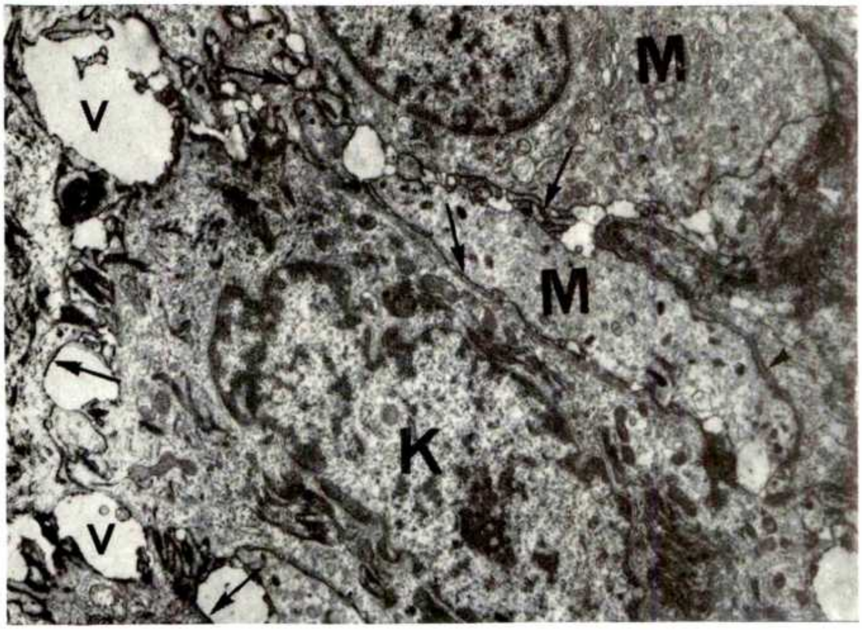

FIGURE 8. Early changes (5 min rubbing) are also seen in the extracellular space near the dermo-epidermal junction. Lanthanum preparation. Lanthanum (arrows) outlines the space and its localized dilatations (V). M, melanocyte; K, keratinocyte; small arrows point to basal lamina (×7800).

in Fig. 2. The intracellular oedema, again most marked at the periphery of keratinocytes, was increased and the extracellular space showed numerous localized dilatations which frequently contained a granular material (short arrows, Fig. 5). The cellular organelles were damaged and distorted, and on a very few occasions vacuoles (approximate diameter 0·3 μm) were seen beside keratinocyte nuclei. They were much smaller than those seen in the previous suction experiments and never appeared to cause nuclear deformation. Some desmosomal attachments were still evident (long arrows, Fig. 5), though membranous rupture and intracellular oedema often made identification of the cell borders difficult. The large intra-epidermal blisters contained in places a granular material devoid of organelles.

Lanthanum staining. Sections from lanthanum treated blocks showed the tracer outlining both the extracellular space and its localized dilatations (Figs. 7 and 8).

DISCUSSION

The light microscopical appearances of established friction blisters reported in this paper, are similar to those reported by other workers (Naylor, 1955; Sulzberger *et al.*, 1966; Fukuyama & Cortese, 1968; Cortese *et al.*, 1969) in that they appear in the upper Malpighian layer. Earlier events, however, seem not to have been recorded previously. This work shows that changes during the pre-blistering phase can be seen provided they are searched for in numerous sections. The electron microscopic appearances suggest that they consist of keratinocyte intracellular oedema, membranous damage and irregular dilatation of the extracellular space. Cell contents then pour out into the extracellular space and the remains of the cells become necrotic. A small intra-epidermal blister (Fig. 1) is formed and this presumably enlarges (Fig. 2) when continuing friction affects neighbouring cells. With further friction the roof of the blister is rubbed off, leaving an erosion.

There is a sharp contrast between the sites of epidermal injury caused by suction and friction. Large paranuclear vacuoles within keratinocytes are an early feature of suction injury, whilst the earliest changes with friction are seen mainly in relation to keratinocyte membranes. The dermo-epidermal junction apparently resists frictional better than suction injury, and as a result blistering occurs at different levels, i.e. intra-epidermal friction blister and subepidermal suction blister.

In dermographism, a frictional force applied to the skin produces a sequence of events probably mediated by histamine release from dermal mast cells. As a result of increased permeability of the superficial dermal vessels, the upper dermis and the epidermis are flooded with increased intercellular fluid. Cauna, Macy & Cralley (1970) have studied skin ultrastructure in dermographic weals. It is tempting to suggest that the changes they noted, which were similar to those reported in this paper, were due to frictional forces, and that the paranuclear vacuoles also reported were due to the increased flow of intercellular fluids (as in suction).

The ultrastructural sites of damage caused by suction and by friction are in turn different from those in other experimental models used for the production of blistering. Low concentrations ($<0{\cdot}4\%$) of collagenase cause a clean epidermal–dermal separation in guinea-pig skin by directly damaging the basal lamina (Kahl & Pearson, 1967b), whilst higher concentrations ($2{\cdot}0\%$) for a longer period produce, in addition, damage to upper dermal collagen. With a short exposure to papain there is dermo-epidermal separation at a level either between the basal lamina and basal cell or deep to the basal lamina, but with a longer exposure there is epidermal acantholysis due to symmetrical cleavage of the desmosomes (Miller & Stoughton, 1960; Kahl & Pearson, 1967a). Cantharidin likewise produces an acantholytic blister within the epidermis, but this is apparently an enzyme-dependent reaction (Stoughton & Bagatell, 1959). Mild to moderate thermal burns, however, produce appearances similar to those we have observed following friction trauma. In both there are intracellular oedema, early loss of organelles, clumping of tonofilaments, and leakage of cytoplasm through ruptured plasma membranes into the intercellular space (Pearson, 1965). Nevertheless, caution should be exercised before inferring that friction damages cells by a local rise in temperature. Naylor (1955), by varying the speed of rubbing and by using heads of different thermal conductivity, concluded that the damage was not temperature-dependent, but more likely due to mechanical distortion of the cells. The observations reported here are quite consistent with such a view.

ACKNOWLEDGMENTS

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British Journal of Dermatology (1974) **90**, 501.

The action of sodium lauryl sulphate on rat skin—an ultrastructural study

P.W.A.TOVELL, A.C.WEAVER, J.HOPE AND W.E.SPROTT

Environmental Safety Division, Unilever Research Laboratory, Sharnbrook, Bedford

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SUMMARY

An assessment was made of the ultrastructural changes in the epidermis of clipped rat skin following six washings over 3 days with 1% sodium lauryl sulphate. Findings included deposition of lipid in most cells, marked spongiosis and vesiculation, partial detachment of basal cells from the basal lamina, isolated rupture of the basal lamina, condensation of tonofibrils within cells of the stratum basale and stratum spinosum, protein deposition between the deeper epidermal cells (epidermal oedema/blister formation) and thickening of the epidermis and stratum corneum. There was a reduction in the number of keratohyalin granules, with no change in their structure or average size. A 1% solution of sodium lauryl sulphate can cause marked general damage to the epidermis of clipped rat skin under the conditions described above.

Sodium lauryl sulphate is an anionic detergent which has been used in a wide variety of products, including shampoos, toothpastes and fabric washing powders. In recent years it has been replaced to a large extent by less irritant detergents, such as ethoxy sulphates and alkyl aryl sulphonates. It has, however, been widely used as a model detergent at a concentration of 1% for irritancy testing (Kligman & Wooding, 1967) and for other biological studies.

The purpose of this investigation was to assess the effect of a high concentration of sodium lauryl sulphate on clipped rat skin, and to note especially any changes induced by the action of the detergent on the keratohyalin granules of the stratum granulosum and on the intracellular tonofibrils of the deeper layers of the epidermis.

There has been no comprehensive study of the ultrastructural changes that take place in the epidermis following superficial *in vivo* detergent treatment (Loomans & Hannon, 1970).

MATERIAL AND METHODS

Eight-week old 100 g female rats of the Colworth/Wistar strain were used throughout.

The dorsal region of each of six rats was clipped. The clipped area of three of the rats was wetted twice daily for 3 days with a 1% aqueous solution of sodium lauryl sulphate (SLS), the remaining three rats being wetted with 0·5% aqueous sodium sulphate as a control. Animals were killed by cervical dislocation.

A small piece of skin from the treated area of each animal was removed, cut into small pieces and fixed in 2% osmium tetroxide in 0·13 M cacodylate pH 7·4 at 0°C for 2 h, prestained in 1% aqueous uranyl acetate for 2 h, dehydrated in alcohol and embedded in Araldite. Ultra-thin sections were post-stained with saturated methanolic uranyl acetate and aqueous lead citrate.

Another small piece of skin from the treated area of each animal was removed and fixed in Davidson's fixative. 4 μm sections were cut from paraffin blocks and stained with haematoxylin and eosin.

RESULTS

After 3 days, treated areas showed signs of coarsening, reddening and oedema.

Electron microscopy revealed several marked and consistent changes in the epidermis of treated animals.

The strata basale and spinosum exhibited general spongiosis with some spongiotic vesiculation (Figs. 1 and 2). The basal cells had become separated from the basal lamina except where the hemidesmosomes linked bridges of cytoplasm between the two (Fig. 2). In places breaks occurred in sections of the basal lamina, the free ends turned up into the epidermis and doubled back on themselves. Many of the spongiotic spaces were partly filled with clearly defined regions of an homogeneous grey granular substance, which occurred to a greater extent in the lower regions of the epidermis amongst the separated basal cells (Fig. 2).

Almost all the cells of the epidermis contained lipid droplets (Fig. 1). The droplets were all of similar size, were not membrane bound and were randomly distributed within the cytoplasm. There were three or less droplets in each cross section of a basal cell making up about 5% of the total cell volume. Cornified cells also contained lipid droplets, which stood out well against the darkly stained background (Fig. 3).

The keratohyalin in the stratum granulosum was reduced in quantity, but there was no marked difference from the controls in size, shape, electron density, consistency or intracellular distribution of the granules. The stratum corneum was thicker than normal, and its cells were tightly packed together. Some parakeratosis and incomplete keratinization was seen (Fig. 3).

The tonofibrils of the deeper epidermal cells had condensed to a concentric band within each cell (Figs. 1 and 2), having about half the radius of the cell and closely resembling isolated preparations of basal cells (Prottey, Tovell & Ferguson, 1974). The basal cell nuclei contained dispersed chromatin, indicative of increased nuclear activity. There was an apparent increase in the number of ribosomes; cross sections of the cytoplasm of some cells consisted almost entirely of ribosomes (Fig. 2). No lymphocytes, macrophages or neutrophils were observed; there was no increase in the number of Langerhans' cells.

In general, optical microscopy confirmed findings from electron microscopy. The epidermis of treated skin was slightly thicker than that of the controls, and the epidermal cytoplasm was more basophilic. Spongiotic vesiculation was clearly seen. The stratum corneum was significantly thickened, and in many places contained nuclear remnants. The stratum granulosum contained less keratohyalin (25–75%). The skin of the control rats exhibited no macroscopic changes, and microscopically appeared normal.

DISCUSSION

The epidermal damage seen macroscopically following SLS treatment presented a consistent pattern of cellular and subcellular changes when the skin was examined microscopically.

The reaction seen in the deeper layers of the epidermis, characterized by intercellular spongiotic vesiculation and oedema, intracellular lipid deposition, and thickening of the strata basale and spino-

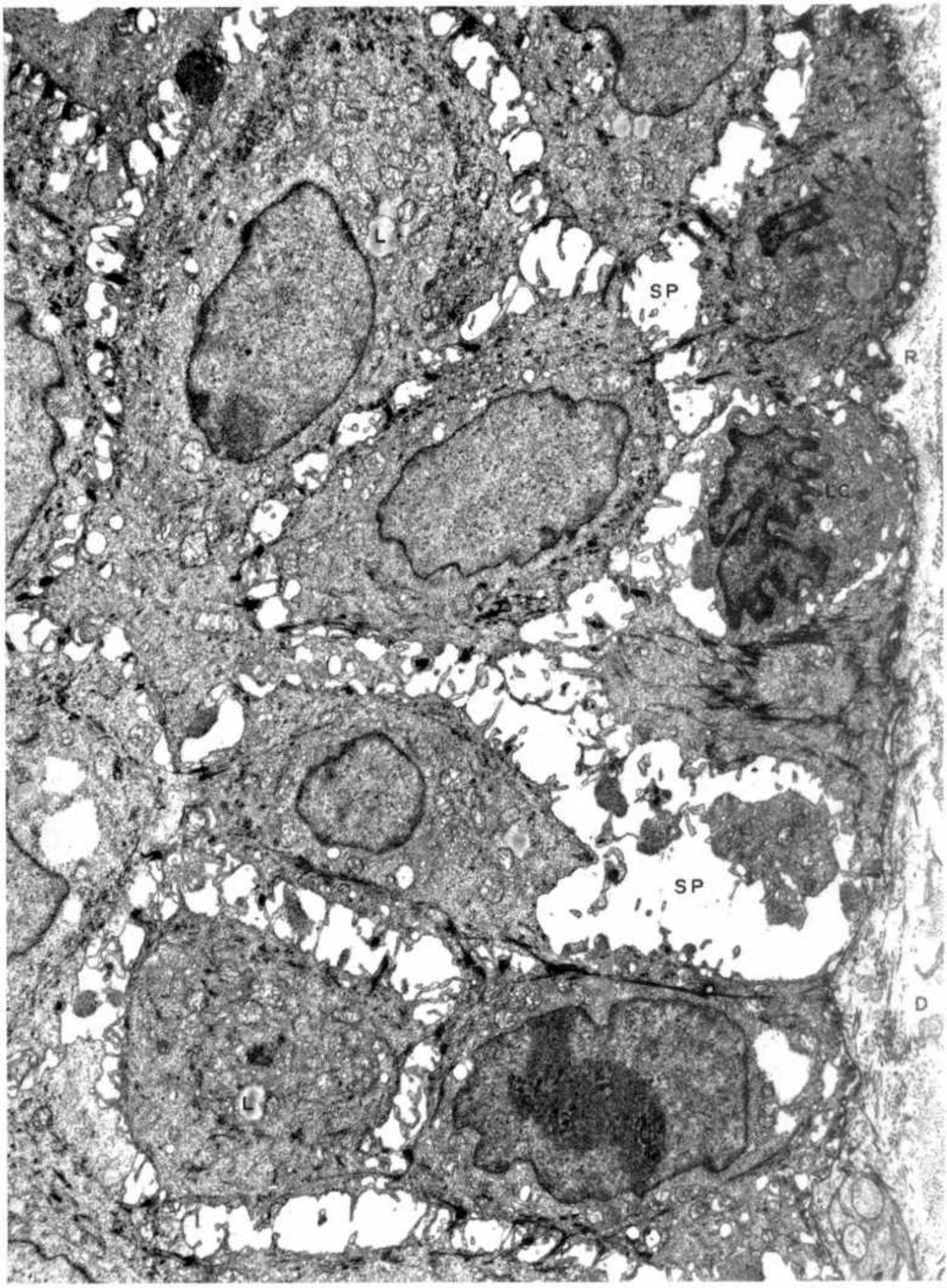

FIGURE 1. Skin treated with SLS, showing spongiosis (SP), a rupture in the basal lamina (R), a Langerhans' cell (LC), condensed tonofilaments, intracellular lipid (L) and dermis (D) (× 7200).

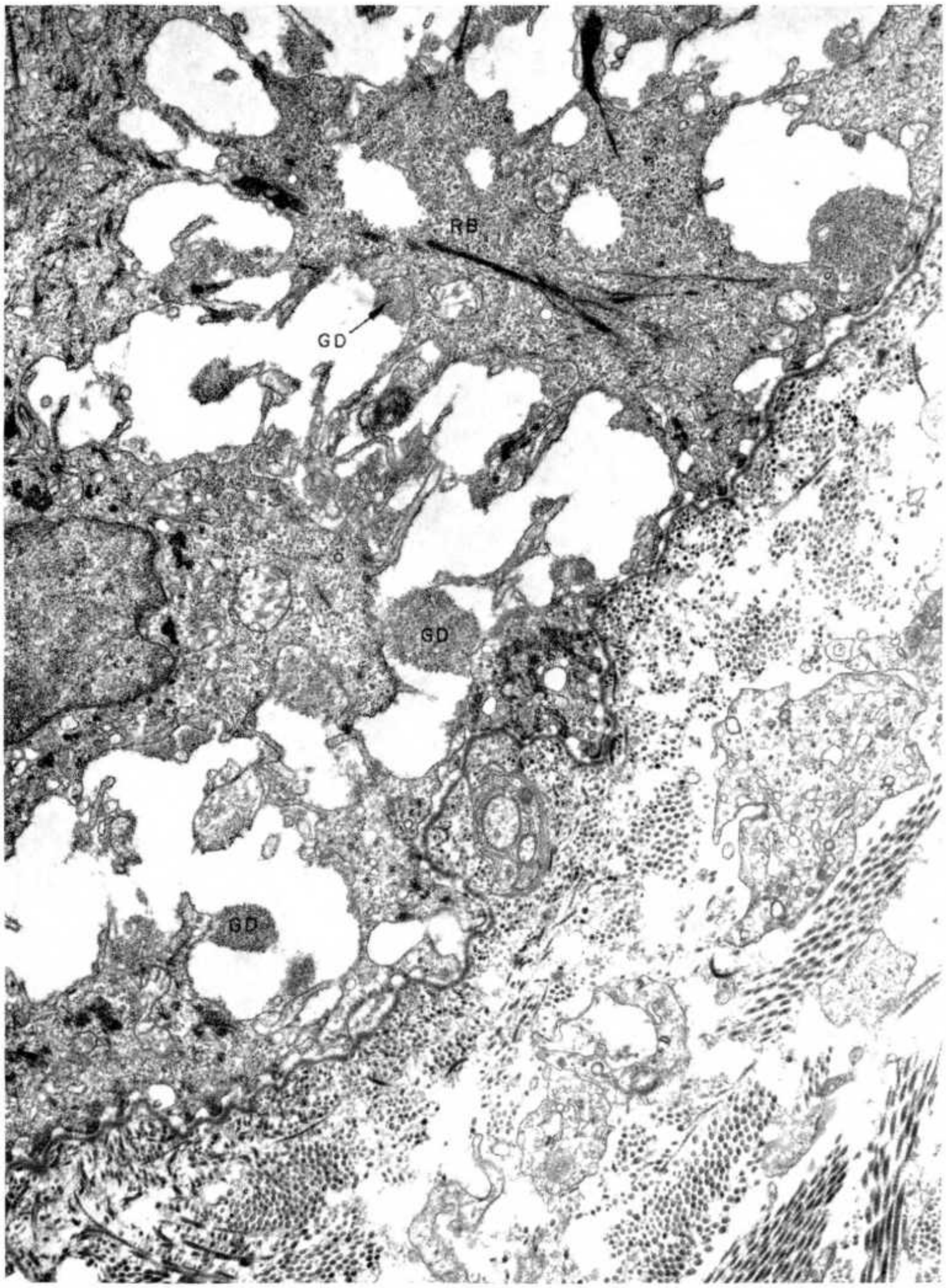

FIGURE 2. Skin treated with SLS. The irregular contours of the basal lamina are clearly seen, as are the granular protein deposits between the cells (GD), and the excessive numbers of ribosomes in some cells (RB) (× 21,600).

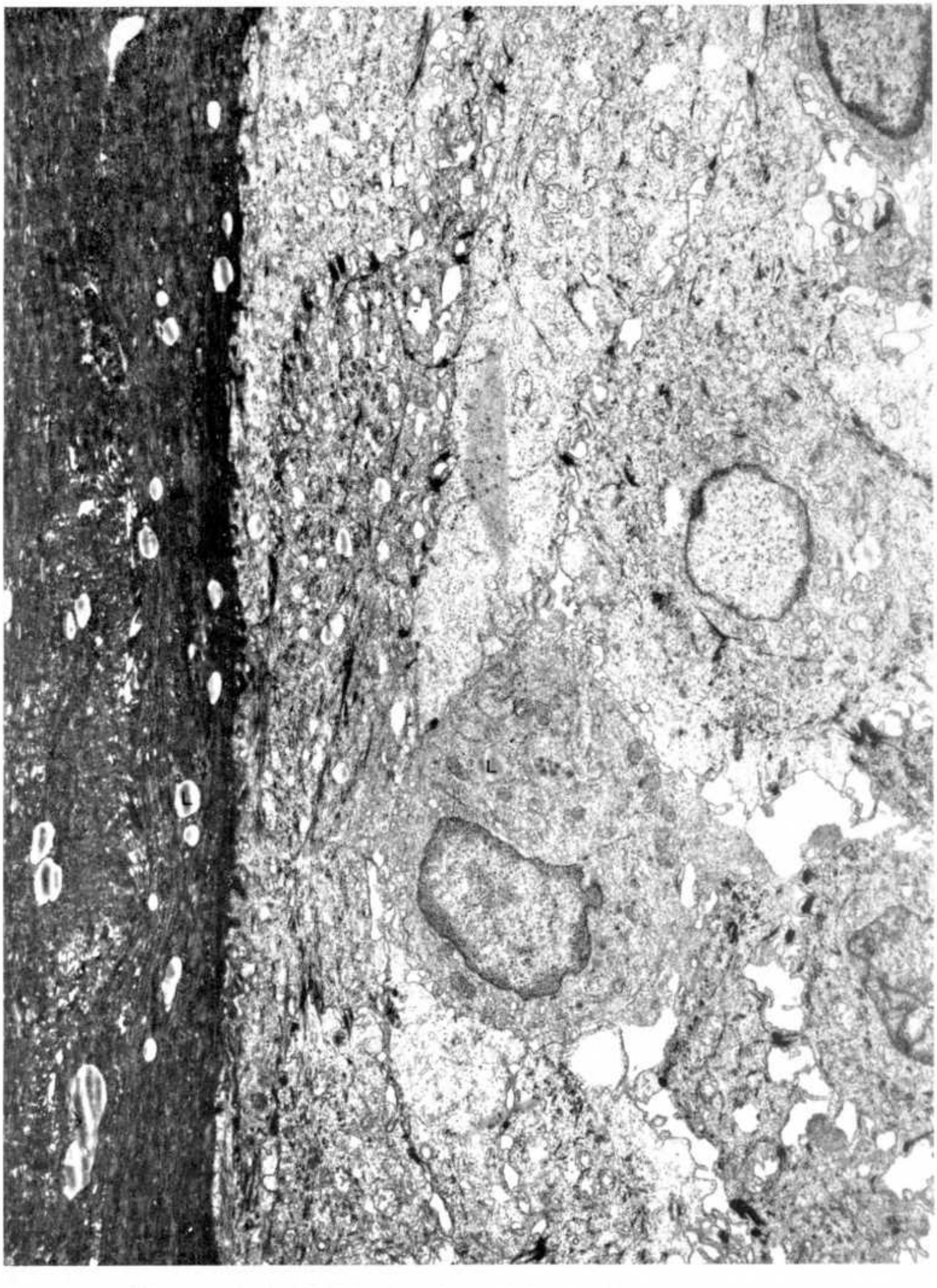

FIGURE 3. Skin treated with SLS showing the lipid deposits (L) in the stratum granulosum and stratum corneum. Note the density of the stratum corneum and the lack of keratohyalin bodies in the stratum granulosum (× 3600).

sum, could reflect cellular hypoxia as a result of direct cell damage or of dermal capillary damage. Under such conditions it would be easier for the cells to metabolize carbohydrate than fat, so that there would be a tendency for intracellular fat to accumulate. Similarly, intracellular fat could be laid down if there was any direct damage to the mitochondrial oxidative processes. Signs of active mitosis, evidence of cell proliferation and the absence of necrosis suggest that SLS acted as a low grade irritant causing sublethal cell damage, whilst at the same time the irritant action stimulated the basal cells to proliferate. These findings are in agreement with those of Prottey *et al.* (1973), who found that rat skin washed *in vivo* with soap or SLS solutions exhibited an increase in DNA synthesis followed by a massive increase in glycero-lipid turnover and accumulation.

The increased basophilia of the epidermal cytoplasm can be accounted for by the apparent increase in the number of ribosomes present—although epidermal cells are normally rich in ribosomes. The spongiotic vesiculation had affected the basal cells to such an extent that they were virtually in isolation, which may explain the similarity of certain cell features to those of isolated basal cell preparations. The irregular contours of the basal lamina were presumably due to the mechanical differences between the points of attachment of hemidesmosomes and the intervening oedematous spaces; breaks in the lamina occurring where the mechanical stresses were too great.

The thickened stratum corneum, the lipid droplets in the corneum cells, the parakeratosis and incomplete keratinization, and the reduced number of keratohyalin granules in the stratum granulosum indicated an acceleration of the keratinization process. This can be interpreted as a protective measure against continued contact with the irritant, and may either have been due to an independent action of SLS on the upper epidermis (Reynold & Tanford, 1970), or have been part of an inflammatory process initiated in the deeper layers of the skin.

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Actinic reticuloid: an immunological investigation providing evidence of basement membrane damage

M.A.MENTER, R.A.McKERRON AND H.E.AMOS

Department of Immunology, Institute of Dermatology, Homerton Grove, London E9 6BX

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SUMMARY

Twelve cases of actinic reticuloid have been investigated using immunological procedures. It was shown that the patients were fully competent immunologically and no evidence was found to support a photo-allergic mechanism in the pathogenesis of the disease. The importance of the basement membrane zone as a site of tissue damage was observed by the finding of a localized band of fibrin deposition in this site using immunofluorescence techniques. Further, it was shown that, by irradiating uninvolved skin at 360 nm, an infiltrate simulating a lymphoma was produced by 24 h. It was concluded that the fibrin deposition was secondary to the infiltrate.

Actinic reticuloid is a disease described by Ive *et al.* (1969). They investigated ten elderly males with photosensitivity extending through the UV to the visible spectrum, and pointed out that the histological picture of the lesions resembled a lymphoma. Other series have subsequently been reported (Heid *et al.*, 1970; Prandi *et al.*, 1972) which support the definite male preponderance and the lymphomatous histology. A careful analysis of Ive's ten cases revealed that the majority of patients had a previous history of eczematous eruptions, associated with erythrodermic episodes often explosive in nature. Different histological patterns were described, but all had intense infiltration of small, round cells, probably small lymphocytes. The association of a long standing tissue damage with precipitous erythroderma and massive lymphocyte infiltration suggested that immunological investigations might be justified.

The work reported in this paper is based on twelve cases of actinic reticuloid not previously included in any published series, and attempted to answer two questions; first, is there a basic immunological abnormality which can precipitate the disease, and secondly, is the disease a result of a hypersensitivity mechanism?

Selection of patients

Patients included in this investigation were attending the Photobiology Department of St John's Hospital for Diseases of the Skin. They were admitted as inpatients specifically for clinical assessment and immunological investigation.

Actinic reticuloid group. Patients previously indexed as actinic reticuloid were examined critically, and those fulfilling the following criteria were selected for the study: (1) males aged between 45 and 70 years; (2) severe, incapacitating, chronic photodermatosis failing to respond to all treatments, including systemic corticosteroids and sun-screens, clearing only on admission to a darkened room; (3) longwave UV sensitivity (320–400 nm), extending sometimes into visible light; (4) histology of exposed areas showing a dense infiltrate of small lymphocytes concentrated mainly in the mid and upper dermis but with focal extension into the epidermis.

Control group 1. This group consisted of eight male patients with known longwave UV sensitivity. It included four with porphyria, and four middle-aged males with longwave UV sensitivity without the histological findings attributed to actinic reticuloid.

Control group 2. This consisted of normal human male volunteers with no previous history of photosensitivity.

MATERIALS AND METHODS

In vivo *investigation—skin testing*

(*a*) *Ultraviolet light.* Patients were tested with a water prism irradiation monochromator (Magnus, 1964). Clinically uninvolved areas of skin were irradiated by a single exposure of 360 nm light using a dose of 5 J/cm^2. Multiple areas were tested, and the induced erythema recorded at 2, 4, 8, 12, 24 and 48 h post-irradiation. Biopsies were obtained for each time interval; half of each was fixed for routine histology and half was frozen in liquid nitrogen for immunofluorescence investigations.

(*b*) *Dermal response to selected allergens.* Patients were tested intradermally with 0·05 ml of mixed bacterial antigen groups 1–5 (Bencard, Ltd) and with PPD 1 : 10,000 (Weybridge). Candidin, Tricophytin and Varidase were also used. Measurements were taken at 20 min, 1, 2, 4, 24 and 48 h.

(*c*) *Photopatch tests.* These were performed on eight of the actinic reticuloid patients as well as on the control group 1 patients. The battery used consisted of bithionol 1%, hexachlorophane 1%, trichlorocarbanilide 1%, trichlorosalicylanilide 1%, tribromosalicylanilide 1%, chlorpromazine (Largactil) 1%, promethazine hydrochloride (Phenergan) 0·5%, sulphapyridine 5%, para-aminobenzoic acid (PABA), N-butyl-4-chlordecylamide (Jadit) 10%, quinine, eth-meth-cinnamate, cyclamate, and fentichlor, each 1%.

In vitro *investigations*

(*a*) *Parameters relating to Type I, II and III tissue damage* (Coombs & Gell, 1968). (i) Total immunoglobulin levels. Total levels of IgG, IgM and IgA were estimated by radial diffusion (Mancini, Carbonara & Heremans, 1965). Commercially available products were used (Hyland Laboratories Inc.).

(ii) Complement activity. The total haemolytic complement activity was determined by the method of Kabat & Mayer (1961) and C3 levels were measured by radial diffusion, again using Hyland products.

(iii) Sera were tested for antibodies reacting specifically against skin determinants, using the technique of indirect immunofluorescence. The substrates used were: involved skin from actinic reticuloid and control group 1 patients; uninvolved skin from the control groups; and skin from control groups previously irradiated at 360 nm. Heterologous substrates were provided by guinea-pig lip and oesophagus. The procedure adopted for immunofluorescence was essentially that described by Coons & Kaplan (1950).

Specific humoral antibodies. Sera were also tested for anti-tetanus antibodies; using standard techniques.

(iv) Immunoglobulin deposition in the skin (direct immunofluorescence). The substrates examined from the actinic reticuloid patients were skin from involved, and uninvolved, purpuric sites and from the areas subjected to 360 nm irradiation. Similarly, irradiated areas both from control groups and from involved skin from control group 1 were studied. FITC conjugates used were anti-IgG, IgM, IgA, complement and antifibrin. The FITC conjugates were obtained from Wellcome Laboratories and the fluorescence assessed using a Reichart Zetospan Fluorescence Microscope fitted with an HBO 200 mercury vapour lamp and using a BG 12/6 mm exciter filter and GG 9/1 mm and OG 1/1·5 mm adsorption filters.

(*b*) *Parameters relating to Type IV hypersensitivity*. Response of peripheral blood lymphocytes to phytohaemagglutinin (PHA) was determined as described by Levene *et al.* (1969). Peripheral blood lymphocytes were cultured with PHA for 72 h at 37°C, and ^{14}C thymidine (Radiochemical Centre, Amersham), was incorporated 24 h before harvesting. The incorporated thymidine was measured in a Packard Tricarb liquid scintillation counter (Model 3315).

Histology

Routine histological examination was done on the involved skin, purpuric skin, uninvolved skin, and specimens taken at various intervals after UV irradiation.

RESULTS

Clinical features of the actinic reticuloid patients

Ten out of the twelve patients were between the ages of 59 and 69; four of them were 61 years of age. The other two were aged 45 and 49 years. Seven of the twelve gave a history of preceding eczema for 10 years or longer. The duration of proven light sensitivity was from 1 to 15 years, six patients having a history of more than 5 years.

The appearance of all patients was remarkably similar, with thickening of the skin over the exposed areas and crusting of the palms (Fig. 1). Unexposed areas were affected to a lesser degree. Purpura was a prominent feature in seven of the twelve patients. This was a lichenoid purpura both on the exposed areas and elsewhere; trunk as well as limbs. Apart from one patient with a carcinoma of the bronchus, there was no evidence of any underlying neoplasia and no lymphoma has so far been detected in this group.

Evaluation of immunological responsiveness

The data obtained on the responsiveness of the immunologically competent cells were within normal limits. The IgG, IgM, IgA and complement levels fell within the normal range (Stiem & Fudenberg, 1966) and all individuals tested were shown to make antibodies to tetanus toxin. Similarly, the lymphocytes from all control and test groups of patients responded normally to PHA stimulation. Further, no evidence was obtained indicating the presence of circulating antibodies directed against either natural or UVR-induced determinants in the skin. Photopatch tests to the defined chemicals were also negative.

Effect of UV irradiation on clinically uninvolved skin

In all the actinic reticuloid patients, erythema developed within 2 h, and a papule gradually developed, reaching maximum intensity between 24 and 48 h post-irradiation. A significant lymphocytic infiltrate

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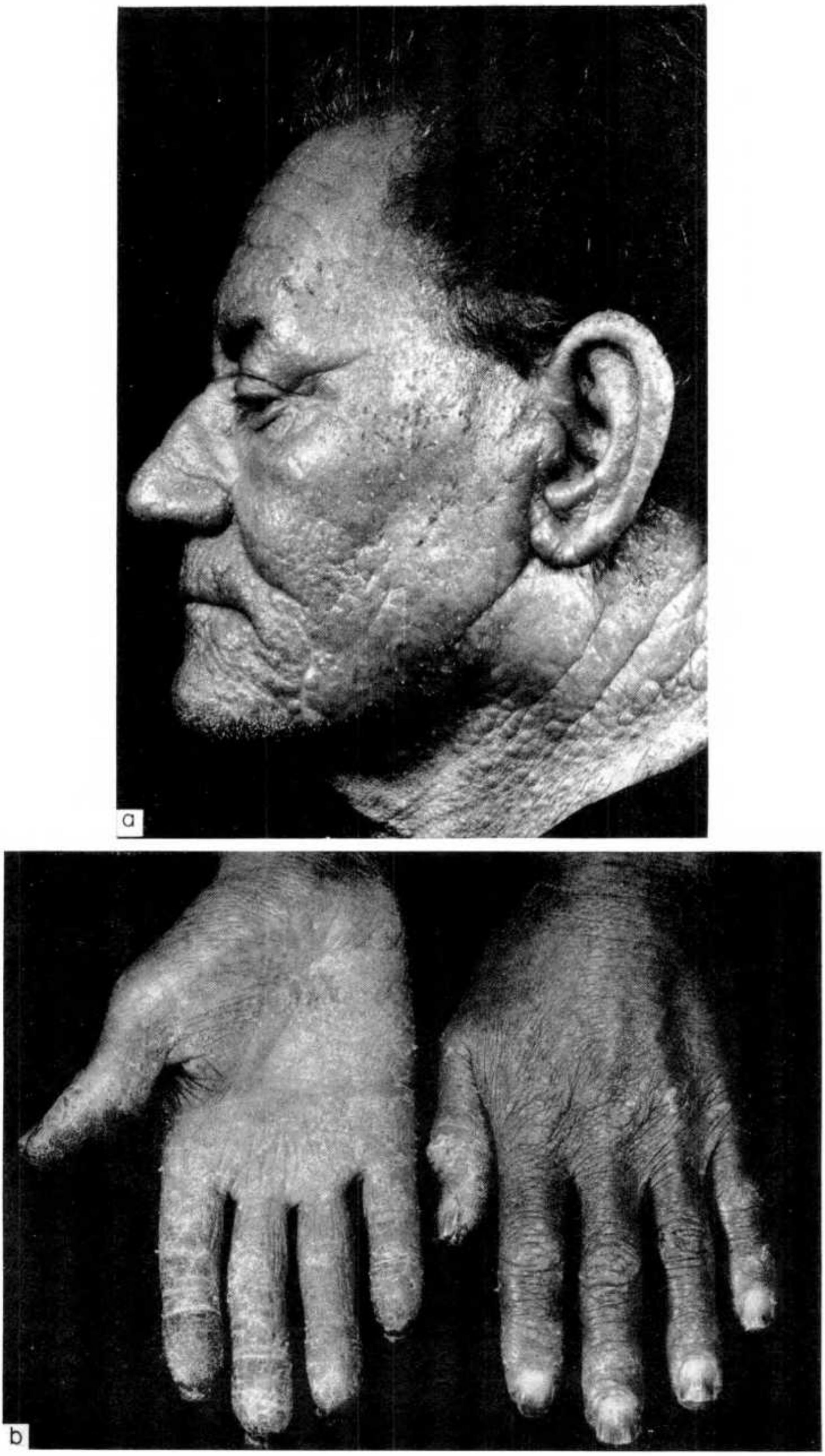

FIGURE 1. Gross appearance of actinic reticuloid. (a) Face, (b) hands.

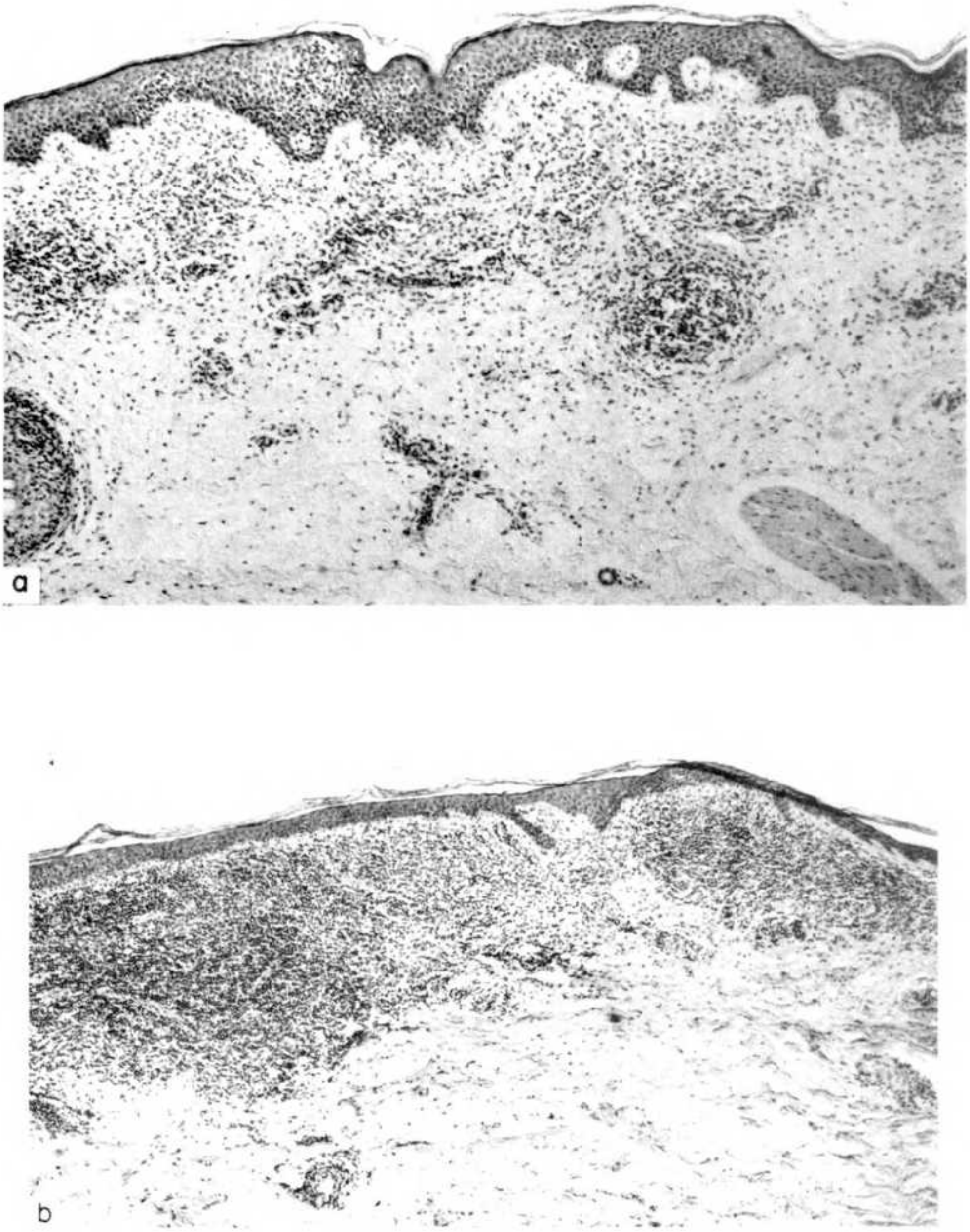

FIGURE 2. Histological appearance following 360 nm irradiation of uninvolved skin. (a) 4 h post-irradiation, (b) 24 h post-irradiation (a, × 32; b, × 26).

was seen in the upper dermis at 2 h in six of the twelve biopsies taken from the actinic reticuloid patients. It was well established in all twelve at 4 h, with focal deposits in the epidermis. It could be seen that the infiltration at 24 h was more marked (Fig. 2).

Direct immunofluorescence investigations

Post-irradiation biopsy specimens. Fig. 3 shows the immunofluorescence pattern in a section of clinically uninvolved skin from an actinic reticuloid patient 24 h after 360 nm irradiation. The staining was confined to those cells in the epidermis which were grouped together in a wedge-shaped area with its apex towards the dermis. Staining was ring-shaped, suggesting a stained cytoplasm or cell membrane. This was detected only with anti-IgG and complement and was not seen in either of the control groups. No fibrin staining was noted.

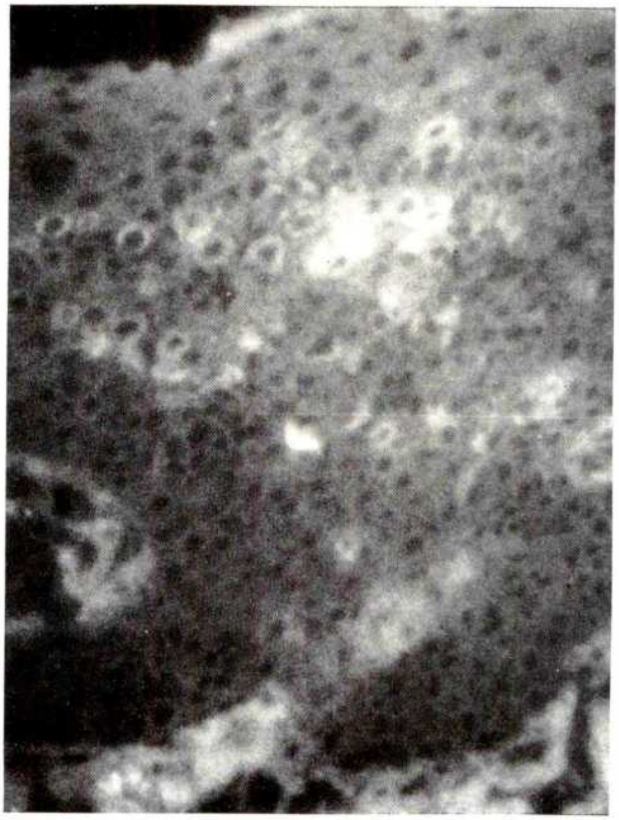

FIGURE 3. Direct immunofluorescence of uninvolved skin irradiated at 360 nm (×250).

Clinically involved skin. The striking finding in involved skin was a definite bright staining with anti-fibrin. The type of staining and its localization is shown in Fig. 4. It was a wide uniform band which followed the basement membrane throughout the length of the specimen. No stain was detected within the dermal papillae or around the blood vessels. This pattern was noted in eleven of the twelve actinic reticuloid patients. It was interpreted as fibrin deposition along the basement membrane. It was not possible to demonstrate any immunoglobulin deposition in the same area using the labelled antiglobulin and complement conjugates. Further, no ring staining of cells in the epidermis, as seen

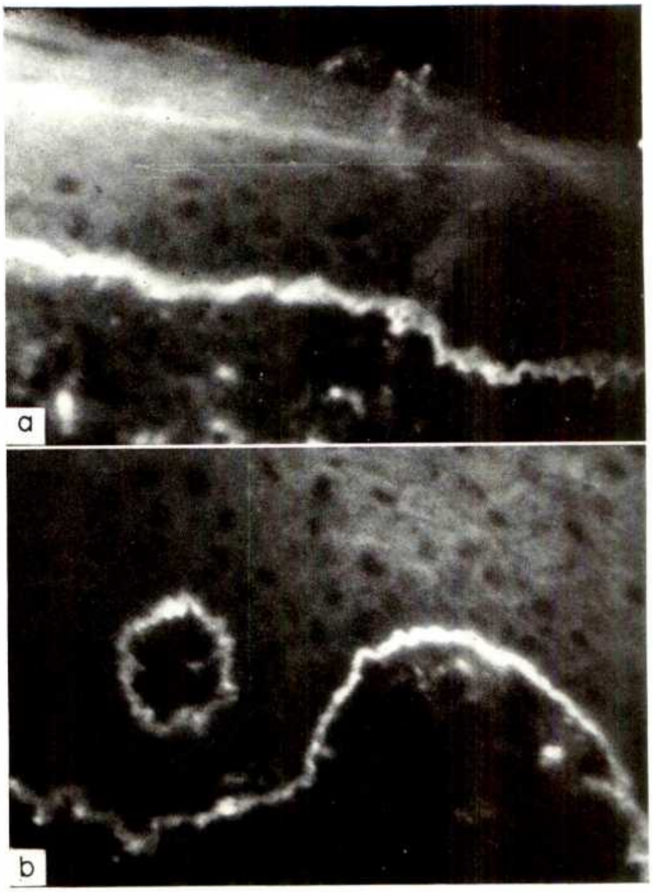

FIGURE 4. Staining pattern with anti-fibrin on involved skin from actinic reticuloid patients (a and b, × 250).

in the irradiated specimens, was detected. None of the control group patients showed the wide uniform staining of the basement membrane with anti-fibrin, although one patient from control group 1 had faint, patchy staining in the same region.

DISCUSSION

The aetiology of actinic reticuloid is unknown. The point had been made, however, that it might be related to a photo-allergic phenomenon, and therefore involve the immunological system (Heid *et al.*, 1970). The twelve patients reported in our series showed no immunoglobulin abnormality and were capable of producing specific antibodies. The response of their peripheral blood lymphocytes to PHA was not significantly different from those from control groups. Thus, patients were capable of mounting both a humoral and a delayed hypersensitivity response to antigenic stimulation. It is therefore unlikely that an immunological deficiency contributes to the pathogenesis of the disease.

Passive transfer experiments have been attempted with sera from actinic reticuloid patients. Positive results have been claimed by Degos *et al.* (1970). They successfully induced a Prausnitz–Kustner reaction with serum from one patient, and using sera from a further two patients, they induced photoreactivity at 48 h post-transfer. This implied that specific circulating antibodies were induced in actinic reticuloid: we were not able to demonstrate antibodies directed against skin determinants using indirect immunofluorescence. This was not too surprising, as the procedure is relatively insensitive, and our attempts to induce antigenic determinants in the skin might not have been successful. We did not feel justified in carrying out passive transfer experiments in view of the known dangers.

The histological picture of actinic reticuloid, we felt, reflected more a cell-mediated hypersensitivity reaction, rather than one induced either by IgE or by other immunoglobulins. Our experiments in which uninvolved skin was irradiated at 360 nm attempted to reproduce a Type IV skin reaction. It was reasoned that if a chromophore was present in the skin, and absorbed at 360 nm, it could be transformed to an allergen to elicit a hypersensitivity reaction. The characteristics of the cell infiltration found in the biopsies of the irradiated lesions, however, did not closely parallel the infiltrate of an allergic contact or tuberculin type of reaction. We demonstrated that, within 2 h post-irradiation, small lymphocytes invaded the area. At 4 h, they formed the majority of invading cells, and had begun to penetrate the epidermis. We were not able to demonstrate either a significant number of polymorphonuclear leukocytes or the biphasic cell response of a Type IV reaction.

The ring fluorescent staining with anti-IgG noted in the epidermis of irradiated lesions is difficult to interpret. No attempt was made to identify the stained cells, so it is possible that they might be related to the invading lymphocyte population which might express IgG receptors on their surface. The localized deposition of fibrin in specimens of involved skin from actinic reticuloid patients point to the basement membrane as a site of tissue damage. Fibrin is found in many skin conditions where there is histological evidence of basement membrane involvement with a chronic inflammatory cell infiltrate, for example lichen planus and lupus erythematosus (Waisman, Dundon & Michel, 1973). The staining pattern in these conditions, however, is more patchy, often granular, and in lichen planus can extend into the dermal papillae. Fibrin deposition in the actinic reticuloid patients studied was uniform in intensity and limited strictly to the basement membrane zone. In view of the relationship of the kinin–complement–fibrinogen systems, it is tempting to reason that the chemotactic factors liberated by activation of these systems were responsible for the cell infiltration. However, it is known that the characterized chemotactic factors, particularly of the complement system, are specific for polymorphonuclear leukocytes (Boyden, North & Faulkner, 1965) and not for lymphocytes. More pertinent to the argument is the finding that the lymphocytic infiltrate could be induced at 2–4 h without demonstrating fibrin staining. This suggests that fibrin is probably laid down secondarily to the cell infiltration.

It has not been our intention to put forward a unifying hypothesis for the aetiology of actinic reticuloid. The data presented showed the patients to be immunologically competent and would not support a photo-allergic mechanism.

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The cell cycle in psoriasis

PETER GOODWIN, SUSAN HAMILTON AND LIONEL FRY

Department of Dermatology, St Mary's Hospital, London, W.2

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SUMMARY

The cell cycle has been determined in eight in-patients with psoriasis. The cell cycle time for the germinative cell compartment has been found to be 91 h, and the turnover time for the epidermal compartment, beneath the keratin layer to be 120 h (5 days). The duration of the S phase (DNA synthesis) has been found to be 10 h, and that for mitosis 30 min. If it is accepted that the turnover time for the keratin layer is 2 days, then the turnover time for the epidermis in psoriasis is 7 days, and not 3–4 days as previously described.

Psoriasis is characterized by a number of changes in the epidermal cell. There is an increase in the total number of cells per unit area of skin, in the number of germinative cells, in the size of the cells (Fry & McMinn, 1968), and in the number of mitotic figures. In addition, the turnover time of the epidermis has been stated to be shorter in psoriasis. Weinstein & Van Scott (1965) reported that, for the epidermal compartment beneath the keratin layer, the turnover time was 2 days, compared to 13 days in normal skin. These observations have not been satisfactorily explained or investigated, but they imply an alteration in the cell cycle of the epidermal cell in psoriasis compared to normal. The cell cycle is the behaviour of a cell during its lifetime and has been defined by Howard & Pelc (1953) as the interval between completion of mitosis and the subsequent completion of mitosis in one or both daughter cells (Fig. 1). There are four recognized components of the cell cycle and it would seem imperative to know how these are altered in psoriasis, as this would undoubtedly help in our understanding of the biological fault. The only previous study of the cell cycle in psoriasis has been that of Weinstein & Frost (1968). The results obtained in that particular study do not correlate with the turnover time for psoriatic epidermis postulated by Halprin (1972). We therefore felt it necessary to determine the cell cycle in psoriasis, using more sophisticated techniques than those used by Weinstein & Frost.

PATIENTS AND METHODS

Eight patients (six male and two female), all over the age of 50, were studied. The nature of the study was explained to them and their consent was obtained.

The technique used was a modification of that described by Brown & Oliver (1968). Using a 26 gauge $\frac{3}{8}$ in. needle, 0·1 ml (10 μCi) of ^{3}H-thymidine (specific activity, 2·2 Ci/mmol) was injected intradermally into the edge of psoriatic plaques on the forearms. Although Fry & McMinn (1970) showed that there was similar cellular proliferative activity in the edge of psoriatic plaques taken from different sites in the same patient at any one time, where possible only one plaque was studied. Great care is required when injecting ^{3}H-thymidine so as to produce uniform labelling. Lapachelle & Gillman (1969) have shown, by comparison between *in vivo* and *in vitro* labelling studies in the epidermis, that DNA synthesizing cells are distributed in a continuous pattern. Only uniformly labelled epidermis has been studied. Eight or more sites were injected at the start of the experiment and skin biopsies using a 4 mm punch and 2% lignocaine as local anaesthetic were performed at set times during the ensuing 24 h.

In vivo ^{3}H-thymidine acts as a 'pulse label' for those germinative cells in the DNA synthesis period at the time of the injection. Any remaining thymidine is rapidly metabolized. Subsequently, the majority of these labelled cells proceed from S phase through G_2 to mitosis. By sampling the tissue over a period of time (in this case 24 h), it was possible to plot a labelled mitoses curve.

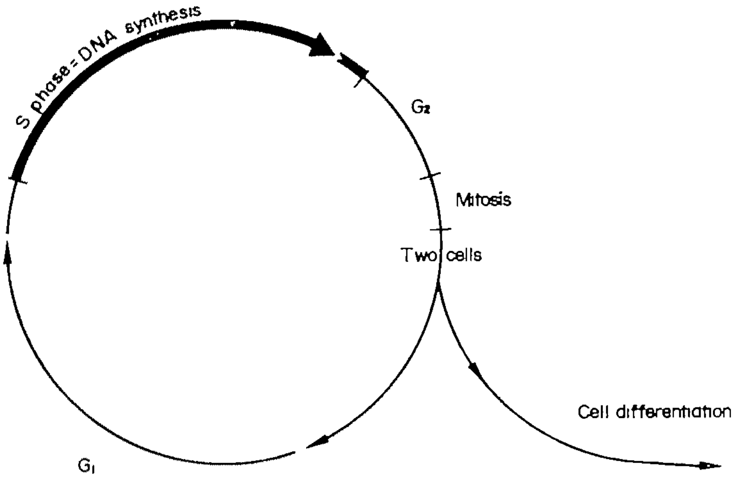

FIGURE 1. Diagram of cell cycle. The cycle has been divided into G_1, the gap between mitosis and the onset of S phase (period of DNA replication), followed by a further gap, G_2, prior to mitosis. At the completion of mitosis the daughter cells may go through another cell cycle or alternatively differentiate. The differentiating keratinocyte is presumed to be a non-dividing cell destined to die without undergoing further mitosis.

Biopsy specimens were prepared histologically in the standard manner. Autoradiographs were made by coating 5 μm sections with Kodak AR.10 stripping film and incubating in light-tight boxes at +4°C for 2 weeks prior to development and staining.

The autoradiographs were viewed under high power (×400). Mitotic figures were counted and then the percentage of labelled mitoses (i.e. number labelled mitoses/total mitoses × 100) was calculated for each biopsy. The average number of mitoses counted in each biopsy specimen of all eight patients was 221 (range 125–309). Because of the difficulty in recognizing early prophase and late telophase figures, only metaphase and anaphase were counted. The labelled mitoses curve was drawn by plotting the percentage of labelled mitoses against time for each of the eight patients.

The durations of the S and G_2 periods were obtained by computer analysis, as described by Steel & Haines (1970). Mitotic duration was calculated from the formula: $N_s/T_s = N_m/T_m$.

The germinative cell cycle and turnover times for the epidermis beneath the keratin layer were calculated from the formulae: $N_s/T_s = N_{gc}/T_{gc}$ and $N_s/T_s = N_l/T_l$; where N_s = number of cells in DNA synthesis, N_m = number of cells in mitosis, T_s = duration of S phase (DNA synthesis period),

T_m = duration of M phase (mitosis), N_{gc} = number of cells in germinative cell compartment (basal cells), T_{gc} = germinative cell cycle time, N_l = total number epidermal cells (not including keratin layer), T_t = turnover time for epidermis.

Analysis of the labelled mitoses curves

The most accurate determination of the cycle can be made by direct measurement of the intermitotic time between the peaks on two successive labelled mitoses curves. Unfortunately, this poses practical difficulties. Biopsies would have to be taken over considerable intervals of time to determine the second peak and many more biopsies would be required. Our method is based on accurate determination of the S phase duration.

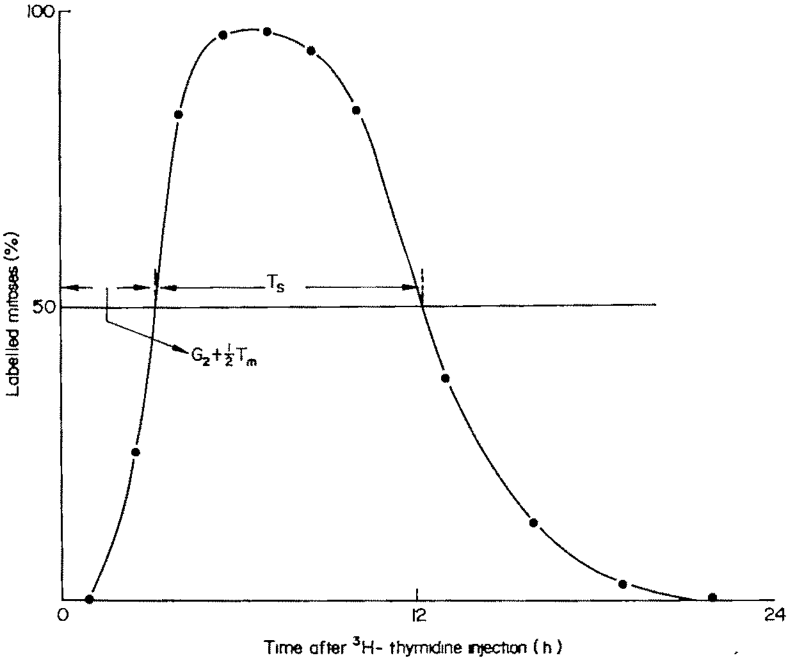

FIGURE 2. Theoretical labelled mitoses curve, showing percentage of labelled mitoses plotted against time in h after ^{3}H-thymidine injection. Because, in reality, the curve never reaches 100%, direct measurement at the 50% level is invalid.

A theoretical labelled mitoses curve is shown in Fig. 2. It can be seen that in the first hour there are no labelled mitoses but that soon after there is a rapid rise; following a plateau region the curve then falls, though at a slower rate than it rose. The average duration of DNA synthe (T_s)sis can be read off direct from the curve as the distance between the 50% points (Wimber, 1963). In addition, the time from the beginning of G_2 until half-way through mitosis ($TG_2+\frac{1}{2}T_m$) is also shown. These measurements are only really valid when the curve approximates to 100%. In practice, it usually fails to do this because there may be a significant number of labelled cells that delay entering mitosis. In addition, because tritium is a weak emitter, theoretically there may be some labelled nuclei deep in the section that fail to expose the photographic emulsion. However, if this is a significant factor, and we do not believe it is, there is no accurate method of calculating this error. This view is supported by Gelfant (1961), who, in his studies on mouse ear epidermis, showed there was no difference between the labelling indices obtained from 2 and 7 μm sections.

Because of the failure of the curve to reach 100% (patient 1 reached 83%, see Fig. 3), a direct measurement of the distance between the 50% points will give an erroneously short result.

In an attempt to improve accuracy, Quastler (1963) recommended reading off the distance at the 37% points or at 50% of the total height of the curve (median 50% level). Our results have been analysed by the method of Steel & Haines (1970), employing the technique of automatic curve fitting. Theoretical curves have been computed on the basis of a model that assumes the phases G_1, S and G_2 are described by independent log-normal distribution. The analysis consists of feeding the computer information to find a labelled mitoses curve that best fits the data supplied.

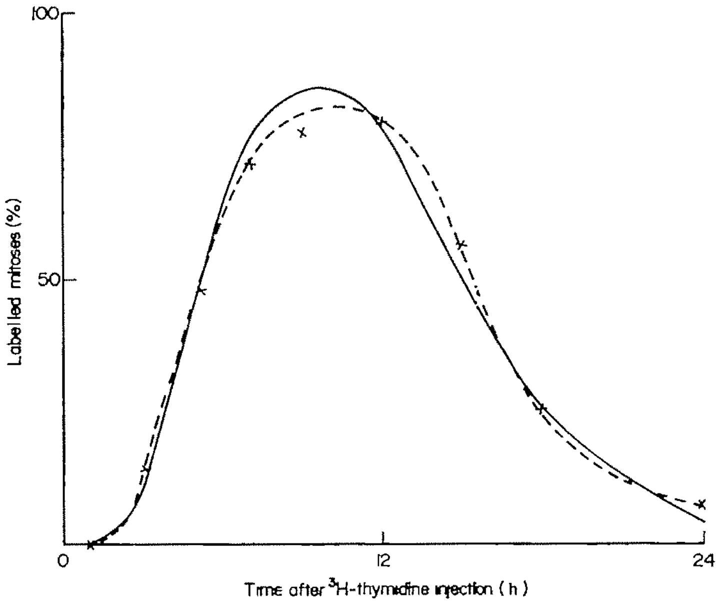

FIGURE 3. Labelled mitoses curve showing percentage of labelled mitoses plotted against time in h after ^{3}H-thymidine injection (patient 1). The original data describes a curve shown by the intermittent line. The continuous line is the computer corrected curve.

Fig. 3 shows the curves obtained from patient 1. The original data describe a hand-drawn curve shown by the intermittent line. The best fit on the theoretical model is shown by the continuous line. The parameters for S and G_2 are not obtained by reading direct from the curve, but are produced by the computer.

Labelling indices

The labelling index is the ratio of labelled cells to germinative cells, i.e. (labelled DNA cells/total germinative cells) × 100.

Definition of the germinative cell population in psoriatic epidermis is difficult and somewhat arbitrary. Whereas in normal epidermis the columnar-shaped basal layer cells are easily recognized, in autoradiographs of psoriatic epidermis they appear as a band of darker-staining cells with an increased nucleus cytoplasm ratio within a zone speckled with labelled cells. To obtain an accurate labelling index, a continuous length of labelled epidermis must be counted. The labelling indices shown in Table 2 are based on average counts of approximately 3000 germinative cells. At the same time, the total number of epidermal cells beneath the keratin layer (N_1, see formula above) was also counted from the same length of epidermis. Counts were made by direct viewing under high power magnification (×400), and not from photomicrographs.

Mitotic index

This is obtained by calculating the ratio of mitotic figures to germinative cells. However, the calculations for mitotic duration require knowledge of the ratio of mitotic figures to labelled DNA synthesizing cells within the same area of epidermis. We have preferred to obtain this ratio by counting 100 high power fields (×400) from autoradiographs for labelled cells, and from adjacently cut haematoxylin and eosin-stained sections for mitotic figures. The mitotic count might have a tendency to be low because of difficulty in recognizing early prophase and late telophase figures. This error will tend to shorten the time obtained for the duration of mitosis.

RESULTS

Cell cycle components

S phase and G_2. The S phase duration obtained by computer analysis varied between 8·7 and 12·9 h (mean 9·8 h). This is significantly shorter than the direct measurement between the 37% points on the hand drawn curves (mean 12·2 h) (Table 1).

The duration of G_2 ranged between 5·7 and 9·0 h (mean 7·2 h) (Table 3).

M phase. Mitotic duration was between 23 and 52 min (mean 30 min) (Table 3).

TABLE 1. To show comparison of S phase (DNA synthesis) duration obtained by computer analysis and an earlier method of direct measurement between the 37% points on the labelled mitoses curve

Patient no.	S phase duration	
	Analysis by computer	Direct measurement between 37% points
1	10·2	12·1
2	12·9	15·5
3	9·9	12·6
4	8·7	10·9
5	9·6	11·9
6	8·9	11·0
7	8·8	11·4
8	9·6	11·9
Mean	9·8	12·2

Labelling indices and mitotic counts

The mean labelling index for the eight patients was 11·0%.

The mean ratio of mitotic counts to labelled germinative cells was 96:1875 (1:19·5) (Table 2).

Cell cycle and epidermal turnover time

In the eight patients studied, the mean germinative cell cycle time was 91 h (range 70–106 h) and the mean epidermal turnover time was 120 h (range 83–157 h) (Table 3).

TABLE 2. Indices used in cell cycle calculations (expressed to 1 decimal place)

Patient no.	Labelling index (%)	Labelled cells: total germinative cells	Mitotic count: labelled cells (per 100 high power fields)
1	11·3	1:8·8	70:1650
2	12·5	1:8·0	118:1726
3	13·4	1:7·5	139:2249
4	12·5	1:8·0	142:2576
5	9·0	1:11·1	40:900
6	8·5	1:11·8	71:1620
7	10·2	1:9·9	96:1989
8	10·9	1:9·2	94:2292
Mean	11·0	1:9·3	96:1875 (1:19·5)

TABLE 3. The parameters of the cell cycle. Mitotic duration (M) is expressed to the nearest minute and the cycle times and turnover times to the nearest hour

Patient no.	S (hours)	G_2 (hours)	M (minutes)	Germinative cell cycle time (Tgc) (hours)	Turnover time (hours)
1	10·2	5·7	26	91	125
2	12·9	7·7	52	104	132
3	9·9	7·7	37	74	90
4	8·7	7·2	28	70	83
5	9·6	6·1	25	106	157
6	8·9	6·6	23	105	151
7	8·8	7·5	25	87	115
8	9·6	9·0	23	88	110
Mean	9·8	7·2	30	91	120 (5 days)

DISCUSSION

These results show that the average germinative cell cycle time in eight patients with psoriasis is 91 h (slightly less than 4 days), the duration of DNA synthesis is approximately 10 h and of mitosis 30 min. These results are considerably different to those of the only other previous study on the cell cycle in psoriasis, by Weinstein & Frost (1968). Weinstein & Frost found the cell cycle time for the germinative cells in psoriasis to be 37·5 h, the duration of DNA synthesis 8·5 h and of mitosis 16 min.

This discrepancy between our results and those of Weinstein & Frost has to be explained. The result which is most similar between the two studies is that of duration of DNA synthesis (the S phase) in the cycle. However, if we had employed the same method of determining the S phase, i.e. taking the distance between the 37% points on the ascending and descending limb of the labelled mitoses curve, our results would have been considerably different. Employing this method, the average duration of the S phase in our study would have been 12·2 h. It has been established that the computer method of Steel & Haines (1970) for estimating the duration of the S phase is a more reliable method than simply taking the distances between the 37% points on the labelled mitoses curve. It would therefore seem that the only explanation for the difference in the results must be in the accuracy of identification of mitoses and of labelled mitoses by the investigator.

Likewise, the estimation of the cell cycle depends on accurate identification and counting of the germinative cells and those which have been labelled in each specimen. One possibility for the differences between the two studies is that Weinstein & Frost did not count their germinative cells by direct viewing from a microscope, but from photomicrographs. This, to our mind, is a less accurate method. In addition, it is likely that, provided the investigator can easily recognize germinative cells, the greater the number counted the more likely will an accurate ratio of germinative to labelled cells be obtained. In our study, on average approximately 3000 germinative cells per patient were counted, whilst the average counted by Weinstein & Frost was 650, and in one patient was only 225. This is probably too low to give a reliable result. If the cell cycle time of the germinative cells in psoriasis is accepted as 91 h, as found in this study, then the turnover time for this part of the epidermal compartment is 4 days. The turnover time for the total epidermal compartment beneath the keratin layer, in our study, was 120 h (5 days), which was the estimated value proposed by Halprin (1972). It is unfortunate that the turnover time for the epidermal compartment has been accepted for many years as 2 days, as this result was achieved by techniques which are not suitable for estimating turnover times. Weinstein & Van Scott (1965), using intradermal ^{3}H-thymidine, noted the time taken for individual labelled cells to reach the keratin layer. In this study the minimal, not the average, transit time of an individual cell in the epidermal compartment was being measured. However, as Halprin (1972) pointed out, the mistake has been made in assuming that this minimal transit time is equal to the turnover time for this compartment, but this is not so. The turnover time is the average transit time of the cells and this cannot be estimated by the technique of Weinstein & Van Scott (1965), as it is known that the movement of cells from the basal layer up through the epidermis is random (Leblond, Greulich & Pereira, 1964), and that they do not move in unison as implied by Weinstein & Van Scott (1965).

The results of our study clearly show that the turnover time for the psoriatic epidermis is considerably longer than the 4 days (2 days for the epidermal compartment and 2 days for the keratin layer) which has been previously accepted (Rothberg, Crounse & Lee, 1961; Weinstein & Van Scott, 1965; Van Scott & Farber, 1971). If we accept that, and add on the 2 days for the turnover time for the keratin layer, our results give a turnover time of 7 days for the epidermis in psoriasis.

It is even possible that the turnover time for psoriatic epidermis is longer than that found in this study. It has always been assumed that the cell cycle is controlled from G_1, i.e. when the need for cell renewal arises, a cell will pass from G_1 to S, and after DNA synthesis, will proceed through G_2 to mitosis. However, Gelfant (1961) has shown evidence for the existence of a separate population of cells. He found that following an injury stimulus to the epidermis, mitotic figures appear sooner than the minimum time taken for a cell in G_1 to pass through S and G_2. He therefore concluded (Gelfant, 1961; Gelfant & Candelas, 1972) that there exist in the epidermis two discrete populations of cells in a G_2 state. One group is thought to comprise cycling cells, whereas the second group remains blocked in G_2 for an indefinite period of time, but still retains the capacity to divide when suitably stimulated (e.g. in response to injury). If the second group of G_2 cells exists in psoriasis, this could account for high labelling indices and erroneously short cell cycle times, because not all the cells synthesizing DNA (labelled cells) will pass through the normal G_2 period to subsequent mitosis. However, if psoriasis is similar to an injury state (i.e. maximally stimulated epidermis), as would appear probable, then it is unlikely that there is this second G_2 population as is thought to be present in normal tissue (Gelfant & Candelas, 1972). Therefore, our estimated cell cycle and turnover times would be correct using the method we employed.

Further work is now required to determine whether the second population of G_2 cells does exist in psoriasis. It would appear likely, however, from the work of Goodwin, Hamilton & Fry (1973), who found that the ratio of labelled cells to mitoses was disproportionately large in the uninvolved (symptomless) skin in psoriasis, that the second population of G_2 cells may exist in this situation. It is possible that in psoriasis the factors which control the cell cycle are operative in the G_2 phase, and not in G_1 as previously assumed.

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Staphylococcus aureus in the lesions of atopic dermatitis

JAMES J.LEYDEN, RICHARD R.MARPLES AND ALBERT M.KLIGMAN

Department of Dermatology, School of Medicine, University of Pennsylvania,
Philadelphia, Pennsylvania 19104, U.S.A.

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SUMMARY

Quantitative aerobic cultures were obtained from fifty patients with chronic plaques, and from twenty with exudative lesions of atopic dermatitis. In the former, *Staphylococcus aureus* was isolated from 90% and the density exceeded $1 \times 10^6/\text{cm}^2$ in 45% of the samples. In the exudative form, *Staph. aureus* was recovered in every case with a mean density of 14×10^6 organisms per cm^2. *Staph. aureus* was the dominant organism not only in the lesions but in nearby clinically normal skin. Despite these high numbers, clinical signs of infection were lacking. Systemic and topical antibiotic therapy produced a profound decrease in *Staph. aureus* density.

It is proposed that high numbers of *Staph. aureus* may aggravate the underlying lesion and be the source of *Staph. aureus* infections in contacts.

Although patients who suffer from atopic dermatitis have been shown to be peculiarly at risk to infection with herpes simplex and vaccinia viruses, overt bacterial infections are not very common (Baer, 1955; Sulzberger, 1971). Local signs of infection, such as suppuration, heat, oedema and crusting, occasionally occur, but systemic signs, such as fever, leukocytosis and lymphadenopathy, are extremely uncommon. Nonetheless, the lesions of atopic dermatitis have been recognized to carry *Staphylococcus aureus*, by students of hospital cross-infection (Hare & Cooke, 1961; Noble & Davies, 1965). For example, *Staph. aureus* was found in fifty-six of 113 patients with eczema (Selwyn, 1965). Only twenty-four of these patients were clinically judged to be infected, and seventeen of the unsuspected carriers served as *Staph. aureus* dispersers. Similarly, in another report *Staph. aureus* was demonstrated in forty-four out of 106 patients with 'eczema' who were judged not to be infected on clinical grounds (Selwyn, 1963). In these studies, no quantitative assessment of *Staph. aureus* density was made. The purpose of this study was to quantify *Staph. aureus* in both the chronic plaques and the acute exudative lesions of atopic dermatitis.

METHODS

Seventy patients ranging in age from 4 months to 19 years were studied. The majority were children between 2 and 10 years, with a prolonged history of the disease. In fifty, the lesions were atypical, pruritic, chronic, lichenified, non-exudative plaques of atopic dermatitis. The antecubital fossa was the site usually sampled. Sixty-six lesions in these fifty patients were evaluated in a quantitative fashion for aerobic bacteria. In twenty-seven, clinically normal skin 5 cm or more away was similarly sampled. At the time of sampling none was using bacteriostatic soaps, or topical or systemic antibiotics. In another twenty, acute exudative lesions were sampled during an acute exacerbation of the disease. Again the majority of lesions sampled were in the antecubital fossa. The afflicted skin showed erythema, oozing, microvesiculation and occasionally slight crusting. Frankly infected lesions were not sampled. No patient had fever, lymphadenopathy or leukocytosis. In fourteen of this group of patients, clinically normal skin 5 cm away was also sampled.

Bacteriological samples were obtained by the detergent scrub technique (Williamson & Kligman, 1965). Single drops from a tenfold dilution series were plated on (1) Trypticase Soy Agar (TSA), a non-selective medium, and (2) TSA with lecithin as a detoxicant and with polysorbate 80 to enhance growth of *Staph. aureus*. In addition, streaks were made on Marshall & Kelsey's medium. The plates were incubated aerobically at 35°C for 2 days. The total aerobes per cm^2 and the proportion comprised by *Staph. aureus* were then determined.

Thirteen of the patients with chronic lichenified lesions were treated with a 1% neomycin cream twice daily for 7 days, followed by bacteriological evaluation on the eighth day. Another eleven received erythromycin, 250 mg four times daily. We sought primarily to compare oral and topical treatment with respect to elimination of *Staph. aureus*. Additionally, we examined the possibility of relating antibacterial efficacy to clinical response. The effect of treatment was evaluated by observing changes in erythema, lichenification and excoriation. Using these indicators, the response was designated as 'improved', 'no improvement' or 'worse'. No other therapy was given during the 1 week of antibiotic therapy.

RESULTS

Cultures from dermatitic skin

In chronic, lichenified plaques the mean density of sixty-six samples was 593,800 organisms per cm^2. *Staph. aureus* was recovered from sixty sites (91%) with a mean density of 403,900/cm^2. In the twenty acute, exudative lesions, the mean density of aerobes was 18,417,000/cm^2. *Staph. aureus* was recovered in all twenty (100%), with a mean density of 15,122,000/cm^2. In both types of lesions, therefore, *Staph. aureus* was overwhelmingly the predominant organism. In chronic plaques, pure cultures of *Staph. aureus* were obtained in nine patients; the density exceeded $10^6/cm^2$ in thirty-one (45%). In the exudative lesions, ten patients yielded pure cultures of *Staph. aureus*; thus exudative lesions carried a tremendously greater number of total aerobes ($t=3{\cdot}77$, $P<0{\cdot}01$) and of *Staph. aureus* ($t=3{\cdot}33$, $P<0{\cdot}01$).

Comparison of normal and dermatitic skin

Staph. aureus was isolated from all fifteen paired cultures of normal and dermatitic skin in the patients with exudative lesions, in twenty-one out of twenty-seven cultures of normal skin, and in twenty-four out of twenty-seven cultures from the chronic lichenified plaques. This difference was not significant. The density of *Staph. aureus*, however, was very much greater in dermatitic skin. Lichenified plaques had a mean density of 554,300 *Staph. aureus* per cm^2, compared to only 1,358/cm^2 on normal skin ($t=6{\cdot}23$, $P<0{\cdot}001$). Likewise, the total aerobic flora was considerably larger in dermatitic skin, 718,900 against 2624/cm^2 ($t=10{\cdot}5$, $P<0{\cdot}001$) (Fig. 1). In patients with exudative lesions, a mean density of 14,505,000 *Staph. aureus* per cm^2 was found, compared to a mean density of 182,352/cm^2 on normal appearing skin ($t=8{\cdot}8$, $P<0{\cdot}001$). Likewise, the total aerobic flora was again considerably larger in dermatitic skin, a mean density of 16,646,428/cm^2 in lesions compared to 232,307/cm^2 in normal skin ($t=9{\cdot}2$, $P<0{\cdot}001$) (Fig. 2).

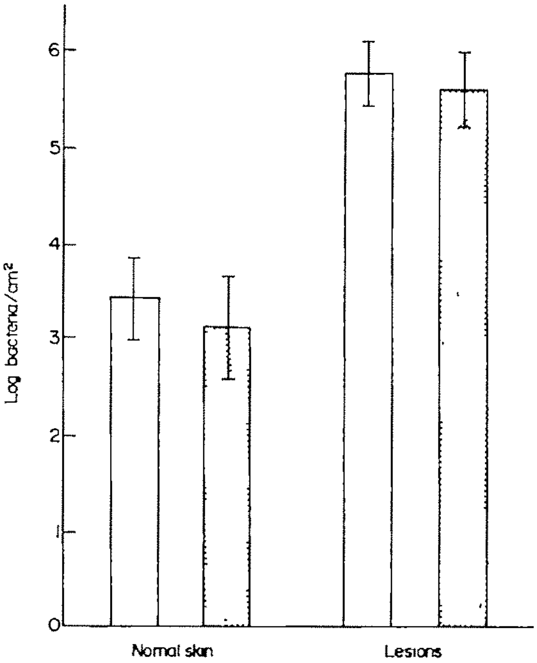

FIGURE 1. Chronic lichenified plaques support both a greater density of total aerobic organisms and *Staph. aureus* than normal skin. *Staph. aureus*, however, is the predominant organism in both normal and dermatitic skin. Open columns, total bacteria; stippled columns, *Staph. aureus*.

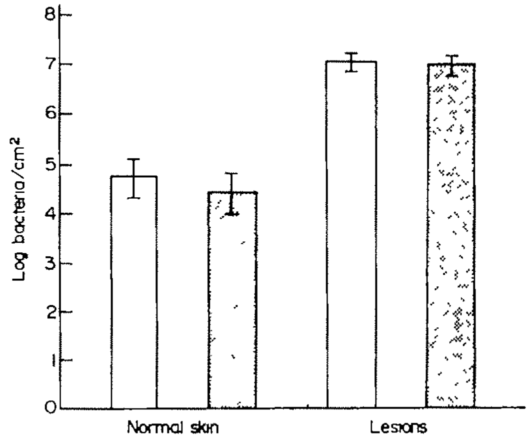

FIGURE 2. Acute, exudative lesions support extremely high numbers of aerobic organisms. Again *Staph. aureus* is the predominant organism. Open columns, total bacteria; stippled columns, *Staph. aureus*.

Effect of antibacterial treatment

All thirteen patients treated with neomycin cream twice daily had *Staph. aureus* prior to treatment, the mean density being 360,000/cm^2 (range 8,421,000–232). Eight had 1,000,000 or more organisms per cm^2; the mean was unduly lowered by four patients who had only 200,000 organisms per cm^2 or less. After 1 week of neomycin, *Staph. aureus* was completely eradicated in six. In the remaining, the mean density was a mere 388/cm^2. The total aerobes fell from 721,000/cm^2 to 342/cm^2. In the eleven treated with oral erythromycin, the mean density of *Staph. aureus* before treatment was 1,700,000/cm^2. After a week of therapy, *Staph. aureus* was totally eradicated in three and the mean count was 1890/cm^2 in the rest. The total aerobic count was reduced from 3,334,000 to 6,180/cm^2 (Fig. 3).

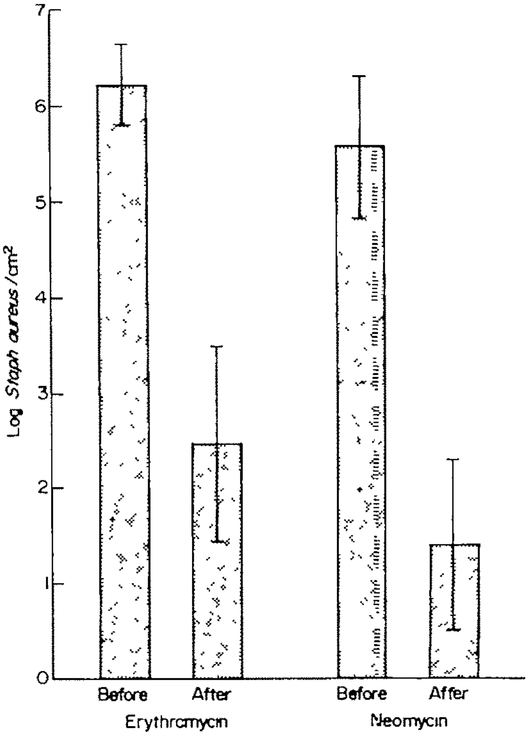

FIGURE 3. Both topical and systemic antibiotic therapy significantly lower the total aerobic flora and the density of *Staph. aureus* in the chronic lichenified plaques.

DISCUSSION

Atopic dermatitis is thought to be a multifactorial disease. *Staph. aureus* overgrowth appears to be a neglected component. More than 90% of patients with chronic lichenified plaques carried this pathogen. Indeed, *Staph. aureus* was overwhelmingly the dominant species with counts exceeding 10^6/cm^2 in about half of the patients. The affinity of *Staph. aureus* for atopic dermatitis was even more pronounced in the acute, exudative form of the disease. *Staph. aureus* was recovered 100% of the time with a mean density of 18,417,000 organisms per cm^2 in acute lesions. In only five of twenty patients was the density less than 10^7/cm^2. Despite these huge numbers, the lesions did not look infected. Only in the acute exudative form was there any hint of a harmful effect of *Staph. aureus*, visible as a slight serous exudate and thin crust.

A question of major importance is whether these extraordinarily high numbers of *Staph. aureus* represent secondary infection or harmless colonization. *Staph. aureus* is not a customary member of the cutaneous microflora except for occasional resident status in the perineum (Allen *et al.*, 1972).

Noble *et al.* recovered *Staph. aureus* nine times in 201 samples from normal skin and in only one was there more than six colonies (Noble, Valkenburg & Wolters, 1967). Retrospective analysis of the clinical notes revealed that those patients who were judged to have improved on the basis of less erythema, fewer excoriations, diminution of serous exudation and a subjective decrease in the amount of pruritis, initially had more than a million *Staph. aureus* per cm^2. This beneficial response was associated with a reduction of *Staph. aureus*. A clinical improvement accompanied by a drastic drop in the quantity of this virulent organism is presumptive evidence for the presence of infection. In our view, which we are still elaborating, secondary infection may be defined in quantitative terms in much the same way as urinary tract infections. Densities above $10^6/cm^2$ suggest that the organism is likely to be aggravating the underlying dermatosis. We refer here to secondary infections only and hasten to point out that antibiotic treatment in such cases produces improvement but certainly not clearing of the dermatitis. The high frequency of *Staph. aureus* in clinically normal skin was also somewhat surprising. Even though the density was low, the recovery rate was the same as for the lesions. It remains an open question whether this reflects continual dispersion from lesions (contamination) or actual colonization with continued multiplication. The uninvolved skin of patients with atopic dermatitis may be inherently favourable to colonization by *Staph. aureus*. Atopic dermatitis, for example, is distinctly different from psoriasis. In psoriatic plaques, the density of *Staph. aureus* uncommonly exceeds 10^3 organisms per cm^2 with a prevalence rate of 50% (Marples, Heaton & Kligman, 1973).

These findings do, however, provide a plausible explanation for the practice of including antibiotics in the initial therapy of atopic dermatitis, even in the absence of the usual signs of infection. Antibiotics are best administered for only a 1 week period, for by that time *Staph. aureus* can be lowered to a harmless level. Prolonged antibiotic therapy may invite colonization with resistant organisms or new pathogens, which could lead to clinical relapse (Leyden & Marples, 1973a, b).

The unexpectedly high numbers of *Staph. aureus* raises the question of how corticosteroids work so well in atopic dermatitis. We are currently conducting a series of experiments with various regimens of local and systemic steroids with quantitative bacteriological monitoring. To date it appears that their action is solely anti-inflammatory; suppression of inflammation leads to unfavourable conditions for *Staph. aureus*. We have not seen any patient develop clinical signs of infection during corticosteroid therapy.

High numbers of *Staph. aureus* may not only intensify pre-existing lesions but may have great importance in relation to public health. Inflamed skin has been recognized as a major source of dissemination of *Staph. aureus* in hospital wards (Davies & Noble, 1962; Selwyn & Chalmers, 1965). Fatal infections have been traced to dermatitic skin. We witnessed one such epidemic of *Staph. aureus* wound infections in a gynaecology ward, the source in this instance being atopic dermatitis in the resident physician. Phage typing and antibiotagram established the identity of the organism in each wound with that of the atopic lesion. On several occasions, we observed primary pyodermas (furuncles) in members of families of patients with atopic dermatitis, the identity of the organism being validated as above.

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In vitro assay of cell-mediated immunity to human wart antigen

WARWICK L.MORISON
Department of Dermatology, St Helier Hospital, Carshalton, Surrey

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SUMMARY

Patients with wart virus infections and control subjects were examined to assess their cell-mediated immune response to wart antigen, using an *in vitro* system, the leukocyte migration inhibition test. It was found that, at the time of resolution of the infection, immunity increased. The possible implications of this are discussed.

The antibody response to wart virus infection in man has been investigated by a number of workers (Almeida & Goffe, 1965; Ogilvie, 1970; Genner, 1971; Pyrhönen & Penttinen, 1972; Pass & Maizel, 1973; Matthews & Shirodaria, 1973). Antibody can be demonstrated in many patients with active or recent infection, but there is no convincing evidence of a correlation between the appearance of antibody and resolution of the infection. The aim of the present study was to determine what role, if any, cell-mediated immune responses play in determining the outcome of a wart virus infection.

PATIENTS AND METHODS

Patients

Blood samples were taken from fourteen patients attending out-patient clinics complaining of various types of warts which had been present for different periods. The mean age of the group was 24 years, range 17–41 years. Children, and patients with ano-genital warts, were excluded from the study. Each patient was investigated on two occasions, at the time of initial presentation and again as soon as possible after successful resolution of the infection. Treatment was prescribed at the first attendance, the procedures used being: paint applied daily (formalin 20%, salicylic acid 20% in spirit), cryotherapy using carbon dioxide snow, or excision.

Controls

Four control subjects were examined; all were free from wart virus infection, although two had a history of warts in childhood. The mean age of the controls was 29 years, range 24–34 years.

Methods

Cell-mediated immune response to wart antigen was measured using an *in vitro* test system, the leukocyte migration inhibition test. The details of this are described elsewhere (Søborg & Bendixen, 1967) but, in summary, it consists of separating the white cells from a venous blood sample and packing them into capillary tubes, which are then mounted in culture chambers containing tissue culture medium and antigen. The chambers are incubated for 18 h, during which time the cells migrate fanwise out of the tubes. The area of migration is mapped out by projection microscopy and measured by planimetry. Results are expressed as a migration index, which is the ratio of the area of migration in the presence of antigen to the area in control cultures. If the subject is sensitive to the antigen, inhibition of migration occurs; an index of 1·0 indicates no inhibition, and values less than 1·0 indicate inhibition and therefore sensitivity to the antigen.

The wart antigen used for this study was prepared as described from surgically removed warts elsewhere (Almeida & Goffe, 1965). The material was examined by electron microscopy using the negative staining technique, and this showed it to be a good preparation estimated to contain 10^{12} virus particles per ml. Preliminary dose–response experiments established that the stock suspension diluted to 10^{-3} gave a satisfactory response in a sensitive patient, without evidence of toxicity in controls.

TABLE 1. Migration indices to wart antigen in leukocyte migration inhibition test as measured before (first) and after (second) resolution of wart virus infection

Case no.	Migration index	
	First	Second
Patients		
1	0·97	0·58
2	1·05	0·55
3	0·93	0·54
4	0·96	0·61
5	0·92	0·63
6	0·91	0·69
7	0·78	0·35
8	1·00	0·60
9	0·94	0·53
10	0·97	0·97
11	0·98	1·03
12	0·85	0·84
13	0·90	0·59
14	0·93	0·38
Control subjects		
1	1·17	
2	1·07	
3	0·98	
4	0·96	

RESULTS

The results obtained in patients and control subjects are shown in Table 1. A migration index of less than 0·8 is regarded as a positive response to the antigen on the basis of work with PPD in Mantoux positive and negative subjects. It can be seen that patients 1–10 and 13 and 14 showed a positive response to wart antigen after their warts had cleared. Three patients failed to show any increase in responsiveness and all four controls were negative.

These results can be compared with the treatments used: patients 1–9 were treated with paint, patient 10 had his solitary wart excised, patients 11–14 were treated by cryotherapy.

DISCUSSION

In vitro measurements of cell-mediated immunity have not so far been applied to wart virus infections, presumably because they are more tedious and require a greater volume of blood compared with antibody studies. However, the latter studies have not provided an answer as to how such infections resolve and, therefore, if an immune mechanism is involved in the resolution, it must be either cellular or a combination of humoral and cellular mechanisms. Some clues can be gained from the clinical situation; clinicians are aware both of an increased incidence and of the intractability of warts in patients with Hodgkin's disease and sarcoidosis. Both these conditions are characterized by defects of cellular immunity. Furthermore, a recent report (Greenberg, Smith & Katz, 1973) suggested that production of contact senstization to 1-nitro-2,4-dichlorobenzene at the site of warts results in a cure of the infection. Presumably, this is due to a greater number of cells concerned with cellular immunity coming into contact with antigen in the warts.

The results of the present study indicate that cell-mediated immunity to wart antigen, as measured by the leukocyte migration inhibition test, increases in some patients at the time of resolution of their wart virus infection. This may indicate that such immunity is involved in the eradication of the infection or, alternatively, the change in immune status may be a result of such eradication.

The influence of the method of treatment on subsequent immunity is interesting; those patients treated with a paint all showed an increase in immunity, while of the four treated by cryotherapy only two showed any change in immunity. These two were treated several times over a period of months, while the two who did not show any increase in immunity required only one treatment. It is possible that successful cryotherapy, like excision, completely separates the antigen from the host, preventing any change in immune status. Furthermore, it could be speculated that paints and unsuccessful cryotherapy have little influence on the natural history of the wart, resolution occurring as immunity develops.

The present study is being extended to include a greater number of patients, to utilize other *in vitro* assays of cell-mediated immunity and to correlate the results with antibody studies. The study has been restricted to adults because of the volume of blood required. Ano-genital warts have been excluded as there is some evidence that the virus involved is antigenically different from the virus of cutaneous warts (Almeida, Oriel & Stannard, 1969).

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British Journal of Dermatology (1974) **90**, 535.

Smodingium (African 'poison ivy') dermatitis

HISTORY, COMPARATIVE PLANT CHEMISTRY AND ANATOMY, CLINICAL AND HISTOLOGICAL FEATURES

G.H.FINDLAY,* D.A.WHITING,† S.H.EGGERS‡ AND R.P.ELLIS§

* Department of Dermatology, University of Pretoria, Pretoria; † Department of Dermatology, University of Witwatersrand, Johannesburg; ‡ National Chemical Research Laboratory, Council for Scientific and Industrial Research, Pretoria; and § Botanical Research Institute, Pretoria, South Africa

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SUMMARY

Smodingium argutum possesses in its sap a C_{17}-catechol which brings about a dermatitis of poison-ivy type. Mass spectrometry shows that American poison ivy, an oriental sumac and Smodingium all produce in differing amounts a series of related compounds which are responsible for skin reactions. History, ecology, botany, histology and treatment are reviewed.

HISTORY

In 1832 Johann Franz Drège, a German explorer, while on an expedition in Pondoland, discovered a woody plant hitherto unknown to science. Not until 1860 were his specimens adequately described and placed in a new genus and species of the Anacardiaceae, *Smodingium argutum*. It seemed to grow plentifully in a rather obscure and wild part of the eastern Cape Province. This territory lies wedged between the escarpment of Lesotho and the Indian Ocean, and comprises the Griqualand East and Pondoland regions.

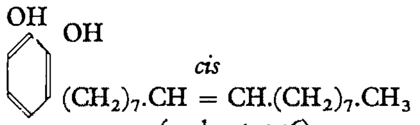
In the early summer of 1912, Sr Stephany, who taught botany at a convent school in Griqualand East, was the first to associate Smodingium with a violent form of dermatitis. Her original findings lay unpublished in the Albany Museum, Grahamstown, where they were later destroyed in a calamitous fire. Half a century later, in 1962, this dermatitis, about which dermatologists knew nothing, was rediscovered by one of us. From some chance clues we traced the whereabouts of Sr Stephany, who is still (1973) alive and aged 94, and obtained her recollections of the original incidents. We were able, from information known mostly to foresters and botanists, to reconstruct the story of Smodingium dermatitis over the 50 preceding years. Folklore, anecdote, witchcraft and scientific observation were duly consolidated in a medical publication (Findlay, 1963). In that paper we gave three reasons for suggesting that the dermatitis produced was of poison-ivy-type: the plant belonged to the same family, the sap was self-melanizing and the skin reactions were closely similar to those caused by poison-ivy.

An enthusiasm for indigenous shrubs and trees had meantime led to the cultivation of *Smodingium argutum* in the Transvaal. It came to be found in many public and private gardens quite beyond its natural range. In the past decade it was established that natural and experimental cross-reactions took place between sufferers sensitive to three plants: American poison-ivy, *Smodingium argutum* and the wax tree, *Rhus succedanea*, an oriental Sumac (Whiting, 1971). Such cross-sensitivity further supported the idea of a chemical relationship.

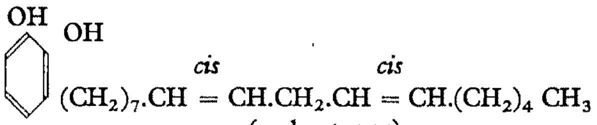
COMPARATIVE PLANT CHEMISTRY

Proof of the close chemical relationship between the allergens from poison-ivy, Smodingium and *Rhus succedanea* has now been obtained. Analyses of Smodingium were made on extracts of large quantities of branches and leaves, supported by more restricted studies on the available material from poison ivy (*Toxicodendron radicans*) and *Rhus succedanea*. Small amounts of the fresh sap from these plants were also collected with a capillary pipette from the secretory canals for mass spectrometry.

The main components of Smodingium sap were two heptadecylcatechols, having one and two double bonds in the side chain.



(mol. wt. 346)



(mol. wt. 344)

Minor components which were not further studied showed: (a) a fully saturated side chain, (b) a side chain with three double bonds, and (c) lower and higher homologues with varying degrees of unsaturation. Details of the chemical investigation, procedures, chromatography, mass spectrometry and nuclear magnetic resonance studies appear elsewhere (Eggers, 1974).

Mass spectrometry of the sap indicated the presence of homologues in various degrees of concentration and unsaturation (Table 1).

TABLE I.

	Molecular weight							
	C_{15} side chain				C_{17} side chain			
	314	316	318	320	342	344	346	348
Smodingium argutum	–	–	±	+++	++	++++	+++	+
Rhus succedanea	–	±	±	+	+++	+++	++++	+
Toxicodendron radicans	+	++++	+++	++	±	±	–	–

These findings suggest that all three species manufacture a C_{15} and a C_{17} side chain type of catechol, in which the predominance of types and degrees of unsaturation varies. Thus, *Smodingium argutum* sap showed large amounts of the C_{17} diolefin, *Rhus succedanea* the C_{17} monoolefin, while poison-ivy itself showed a prominent diolefin at C_{15}, and traces of C_{17} side chain compounds.

PLANT ANATOMY

The noxious sap of Smodingium moves in a system of prominent intercellular secretory canals within the plant body. Whether a species of the Anacardiaceae is toxic or not, the pattern of these canals is much the same (Metcalfe & Chalk, 1950). In all likelihood the spaces are formed around a cleft which is lined with secretory cells. Canal formation by cell lysis occurs only doubtfully.

In the stem of Smodingium, there is a single large secretory canal in the primary phloem of each vascular bundle (Fig. 1). External to the vascular bundle lie firstly the sclerenchymatous pericycle, and then the outer cortical parenchyma, neither of which contains secretory canals. There is no evidence of canal formation in the secondary phloem, though one supposes that they may be found

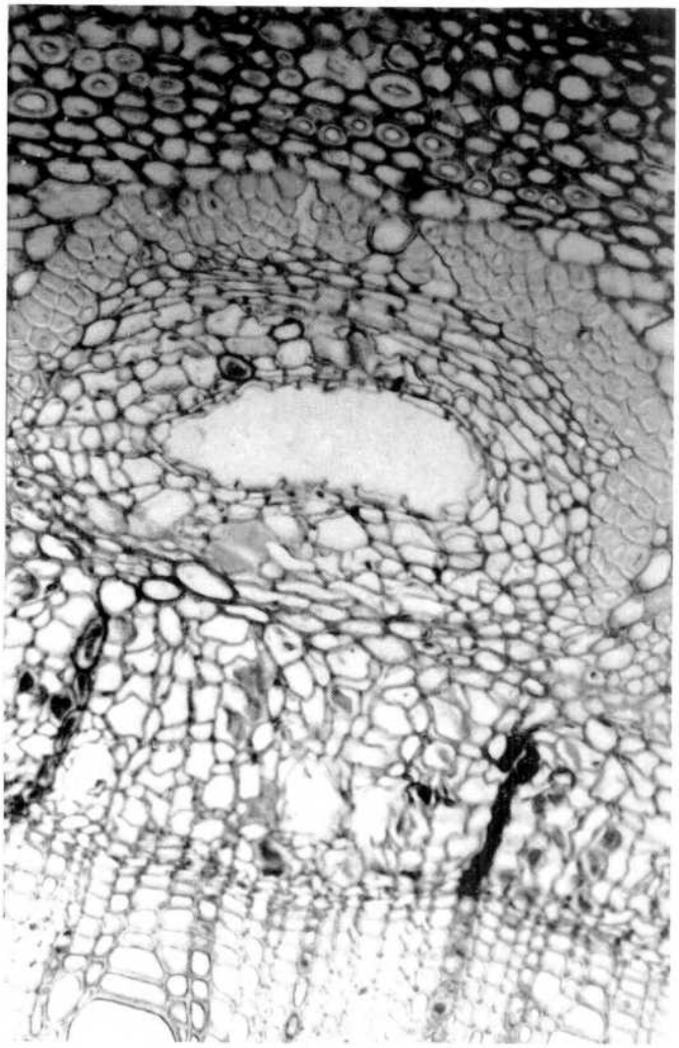


FIGURE 1. The intercellular secretory canal in the primary phloem of the stem of Smodingium seen in transverse section. Above the canal is the horseshoe-like pericycle (× 32).

in older material. Interior to the primary xylem, in the pith, there is a ring of smaller medullary resin canals. Pyrocatechol tannins were not observed.

In the petiole, i.e. leaf-stalk (Fig. 2), there is a ring of fine secretory canals seen in transverse section, with the largest one situated adaxially; arc-shaped pericycle caps lie exterior to the canals. In the centre there is the heart-shaped column of xylem tissue. As in the stem, the canals are therefore also located in the phloem. The bundles and canals branch when reaching the leaflets.

In the leaf, the midrib includes a ventral band of three bundles with secretory canals surrounded by pericycle arcs. Dorsally there is a single bundle (cf. *Rhus diversiloba*, which has five to seven bundles arranged in an arc, McNair, 1918). Scattered throughout the midrib there are small irregular secretory canals. All the lateral veins of the leaf contain at least one secretory canal in the phloem on their dorsal sides. Some canals end blindly in the mesophyll and others anastomose in a reticulate manner, as do the bundles which they accompany. For further details see Ellis (1974).

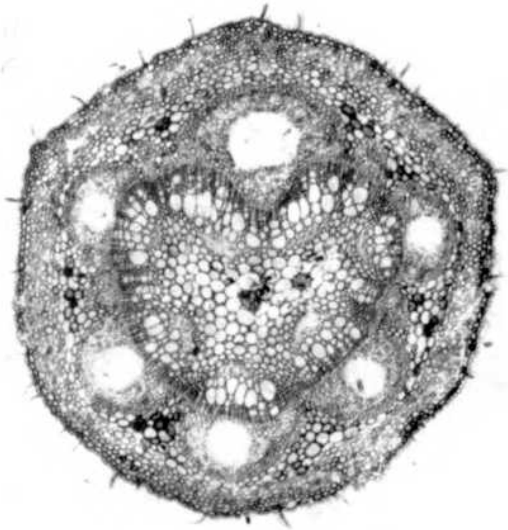


FIGURE 2. Arrangement of secretory canals in the transverse section of the Smodingium petiole, the largest being placed adaxially at the top of the heart-shaped xylem (× 5).

SMODINGIUM SAP: FUNCTION AND TRANSFER

When first expressed from the canals, the resinous sap is transparent or light grey, but on contact with air it rapidly becomes creamy, coagulates, and hardens into a brown or black varnish-like mass. When fresh, the sap under the microscope shows minute globules in a colourless liquid. The globules then darken, and at the same time rectangular crystals separate out. Only two suggestions have been made on the coagulative function of the sap: one is that a protective scab is quickly formed when the plant is wounded, and the other is that the resin canals become obstructed by enlargement of the parietal cells at the base of the petiole, thus enhancing leaf fall.

The petioles, midrib and veins of the leaves are fragile, and pruning of the stems also spatters the sap. Droplets therefore readily escape from the secretory canals and become streaked and scattered on the skin. It has even been alleged that a high wind can break the leaves and spray the sap droplets on a passer-by, who has not even touched the tree. The sap also spreads in a layer on the surface of water; we have seen two cases of dermatitis, sock-like in distribution, in Smodingium-sensitive boys after dangling their feet in water into which they had thrown some Smodingium branches. Sap transfer has also been known through contact with solids, e.g. clothing, tools.

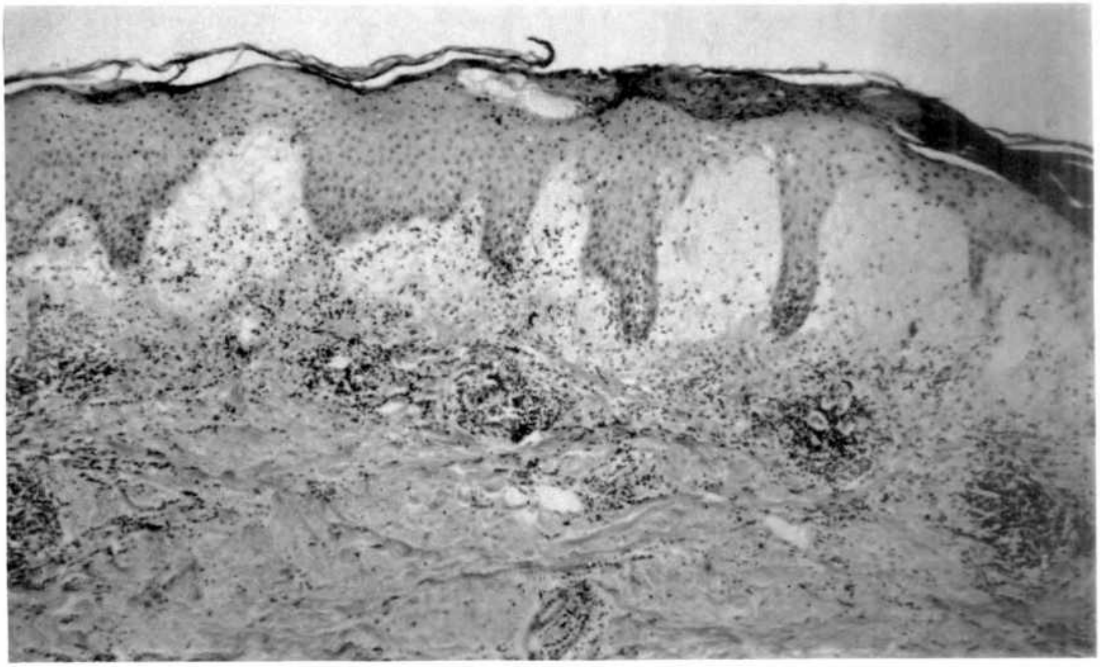


FIGURE 3. Histology of a subacute dermatitis from Smodingium sap, showing superficial coagulative necrosis, some necrobiosis in the mid-epidermis, papillary oedema and upper dermal lymphocytic–mononuclear infiltrate around the vessels.

DERMATITIS

Smodingium dermatitis is not common in consultant dermatological practice, the frequency varying from 0·1 to 0·2% of all new cases seen. Over the past 10 years we have seen thirty-five such patients. With few exceptions the plant responsible grew in a private garden, though not always at the patient's own home. We also know of outbreaks among the pupils of three different Transvaal schools, from Smodingium bushes and trees in the playground, forty children being affected in all. Two cases were traceable to trees in a public park.

The usual mode of contact with the plant was of an unthinking, incidental kind. Most of the contact took place during play, in the course of walking or working about the garden. Direct picking, pruning or breaking of the plant was less important. Only one instance of a work injury was seen, in which an electrician, while repairing some outside wires, was affected by a Smodingium creeper on a customer's house. The head, neck and limbs were the regions on which the dermatitis most usually appeared, related obviously to the parts mostly uncovered, though some 15% had lesions on the body through contact while wearing only shorts or bathing trunks. In one instance there was transfer of sap by fingers to the external genitals.

About one-third of the cases can be described as severe—erythema with oedema and blistering, and as a rule with some evidence of streaky, angulated and spattered lesions. The rest were mild and localized, though often mysteriously recurrent in character. Lacking a test extract which was pure, safe and reliable, we were content to make the diagnosis once the nature of the dermatosis and a proven source of contact were established. Patch tests with the leaf were strongly positive in all seven cases so tested. In three cases each, a simultaneous positive patch test result was obtained with *Rhus succedanea* and *Toxicodendron radicans.* As found in our earlier studies, there was no sparing of age, sex or race, provided the chance for contact existed. The school epidemics emphasized the high sensitizing potential of the plant. As it is deciduous, most of the casual cases occurred from contact with Smodingium leaves in summer, but trouble which followed winter pruning indicated that the stem sap was also liable to cause dermatitis.

SKIN HISTOLOGY

Six skin sections were available (five from Smodingium dermatitis and one from a *Rhus succedanea* reaction). Epidermal changes were of four types: (a) large, well demarcated, bloated vesicles extending through practically the full epidermal thickness and containing coagulum and some lymphocytes; (b) diffuse and patchy dissolution of epidermal cells and their nuclei in the mid-epidermis and rete ridges, with spongiosis, cell shrinkage, a confluent loss of definition and pigmentary incontinence; (c) superficial circumscribed areas of coagulative epidermal necrosis lying like saucers in the superficial epidermis in continuity with the surface; (d) regenerative changes tending towards early surface rejection of the lesions noted under (c). The papillary dermis showed intense oedema without cell infiltration or haemorrhage. This oedema was absent altogether in those areas where the epidermis had started to regenerate. In the upper dermis there was perivascular oedema, with a fairly dense lymphocytic–mononuclear infiltrate around the vessels. There was mild capillary-wall thickening. Only an occasional eosinophil was seen. There was some cytoplasmic but no nuclear debris. There was some infiltration of inflammatory cells into the necrotizing rete ridges. To summarize, the intensity of the damage declined from the surface inwards and ceased in the mid-dermis, presenting successively epidermal necrosis, epidermal necrobiosis, dermal oedema and cell-infiltration. Regeneration occurred with the disappearance of oedema and lessening of the cellular infiltrate (see fig. 3).

TREATMENT

Treatment of Smodingium dermatitis has always begun with identification and avoidance of the plant, and proceeded from antipruritic lotions and oral antihistamines in mild cases to systemic corticosteroids for the severe ones. Improvement has not been particularly rapid. One supposes that the catechol remains in the epidermis until the contaminated layers are shed.

ACKNOWLEDGMENTS

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Photo-contact dermatitis arising from the presence of quindoxin* in animal feeding stuffs

K.W.SCOTT AND T.A.J.DAWSON

Department of Dermatology, Craigavon Area Hospital, Craigavon, Northern Ireland

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SUMMARY

Seventeen patients suffering from photo-contact dermatitis due to quindoxin sensitivity are described. The source of contact was animal feeding stuffs.

A number of substances, when added to animal feeds in a concentration of the order 50 mg/kg, significantly increases the rate of growth of the animals given such feeds. Some of the substances concerned are antibiotics, and it is possible that they may exert their effect by modifying bacterial populations. This is much less certain with many other substances capable of promoting growth, such as copper sulphate and arsenilic acid. The subject has been reviewed by Lucas (1972). Though the addition of such growth stimulants to animal feeds has been widely practised for many years, reports of their producing disease in man are extremely rare. Von Melhorn & Beetz (1971) recorded a single case of contact dermatitis due to sensitivity to ethoxyquin used as an anti-oxidant in animal feeds. Their patient was employed in the manufacture of compounded animal feeding stuffs. Neldner (1972) reported two cases of allergic contact dermatitis due to additives in animal feeds. Tylosin was the source in one patient, and, in the other, patch-tests with additives containing nitrofurazone and tylosin were strongly positive.

In April, 1971, quindoxin (quinoxaline 1,4-di-N-oxide) was introduced in Northern Ireland for addition to animal feeds as a growth promoting factor. The concentration in the feed as offered to the animal was of the order of 20–50 mg/kg. Quindoxin has the structural formula shown in Fig. 1. Chemically it is distinct from quinolines, including ethoxyquin. It was sold under the name of 'Grofas'.

Following the introduction of quindoxin into this area as a growth stimulant, cases of an unusual dermatitis began to appear among workers engaged in the agricultural industry. All the patients suffering from the dermatitis had been handling animal feeding stuffs. A preliminary report describing five such patients, and identifying the allergen as quindoxin, was published in 1972 (Dawson & Scott). The purpose of this article is to report, in more detail, a further seventeen patients. Seven of the patients in the present series were male, and ten female. With one exception, all the patients were engaged in pig farming, and by far the greater part of their work comprised breeding and rearing piglets. The single patient not directly engaged in pig farming was employed delivering supplies of animal feeding stuffs by lorry.

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* We understand that since this article was accepted for publication, Imperial Chemical Industries Ltd have withdrawn quindoxin from the market, as investigations carried out in their laboratories indicated that it could be carcinogenic to experimental animals when fed to them in high dosage.

CLINICAL FEATURES

Clinically, the rash was strikingly similar in each case. The lesions were more marked on light exposed areas though, in some patients, less obvious lesions were present on covered areas. The affected parts were the site of a rather dry eczematous dermatitis which showed a strong tendency to develop fissures. The general appearance in many respects resembled cement dermatitis. Characteristic lesions on the hands are shown in Fig. 2.

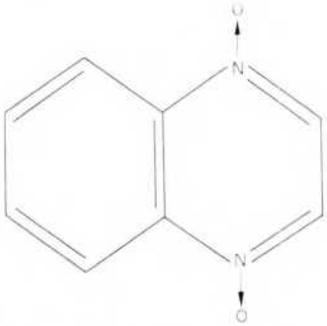

FIGURE 1. Structural formula of quindoxin (quinoxaline 1,4-di-N-oxide).

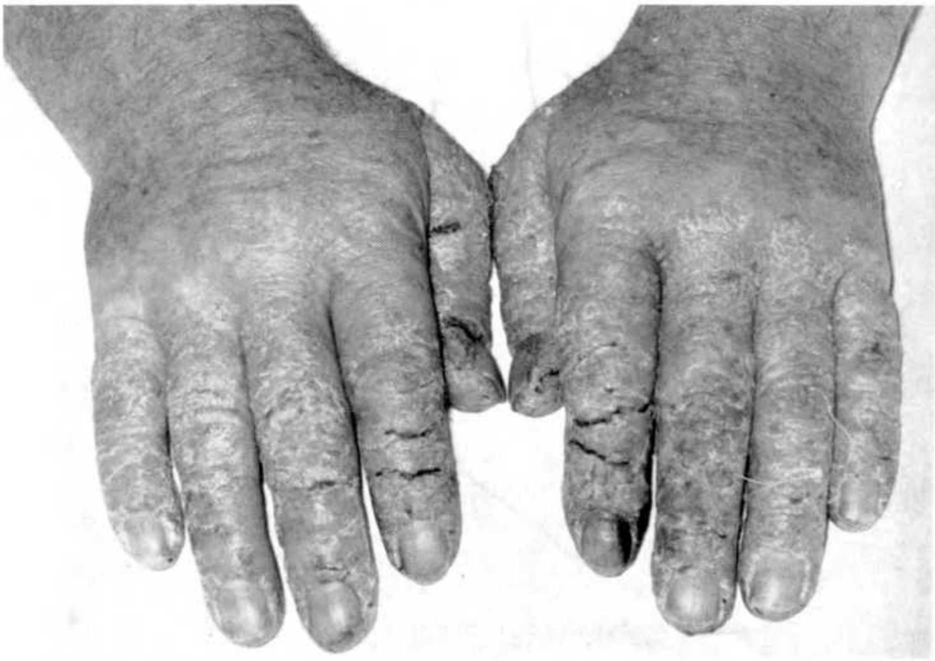

FIGURE 2. Photo-contact dermatitis due to quidoxin sensitivity affecting the hands.

PATCH-TESTS

As the distribution of the lesions and the preliminary study (Dawson & Scott, 1972) indicated that photosensitization was at least in part involved, photo-patch-tests as well as closed patch-tests were included in the present investigation.

The technique employed for photo-patch-testing conformed to the principles summarized by Hjorth & Fregert (1968). The ultraviolet source used was a mercury vapour arc lamp, and in each case the minimal erythema dose (MED) was determined before beginning the test. Approximately half the MED, as determined for each individual, was then used for irradiating his or her test site. Photo-patch-tests were removed and irradiated at 24 h, closed patch-tests were removed at 48 h. Both types of test were read at 48 h and 72 h. All patch-testing was carried out on the patient's back.

Photo- and closed patch-tests were carried out with quindoxin, ethoxyquin and copper sulphate. Tests were carried out with ethoxyquin as this substance was known to be widely used as an anti-oxidant in animal feeds, and with copper sulphate as this was known to be added as a growth stimulant. The quindoxin patch-tests were carried out with quindoxin 0·1% in yellow soft paraffin (BP). Various dilutions were tried, but it was concluded that this was the most satisfactory concentration and vehicle for routine use. This concentration is much higher than the 0·01% suggested in the report on the five original patients. Ethoxyquin was employed as Santoquin (ethoxyquin 66 parts; carrier 34 parts) 1% in yellow soft paraffin (BP); copper sulphate as 1% aqueous solution. Appropriate controls were applied in all cases.

A battery of twenty-two common allergens was also applied as standard closed patch-tests.

All seventeen patients gave strong positive results with the quindoxin photo-patch-test at 48 h. Seven patients gave a positive result and ten patients a negative result with the closed quindoxin patch-test at 48 h. Of the ten patients who gave a negative result with the closed quindoxin patch-test at 48 h, eight were available for review at 72 h. Positive results were found to have developed at the closed quindoxin patch-test sites in each of these patients.

No positive results with copper sulphate or ethoxyquin were obtained.

The tests with the battery of common allergens produced eight positive results distributed among seven of the seventeen quindoxin sensitized patients. Six patients each developed a single positive reaction to balsam of Peru, benzocaine, chlorquinaldol, epoxy resin or wood tar. One patient developed positive reactions to both chinoform and chlorquinaldol.

In four patients, in addition to the seventeen already mentioned, a negative result was recorded with quindoxin both on closed and on photo-patch-testing (concentration 0·5% in two cases, and 0·1% in two cases). Three of these patients were available for review a week later and in no case was iatrogenic sensitization observed. On reviewing the histories and clinical findings in these four patients, it became clear that none of them was in fact suffering from contact dermatitis due to quindoxin sensitivity.

In five patients already diagnosed as having a contact allergy to quindoxin, further patch-tests with quindoxin 0·25% in yellow soft paraffin (BP) were applied under three layers of a black textile, which had been tested for light opacity against a 60 W domestic electric light bulb. The patches were removed and the sites inspected at 24, 48 and 72 h. In two patients only, was a very faint reaction observed at 72 h under the black textile covered patches. In all these cases, strong positive reactions developed at all sites within 24 h of removing the black textile patches. It therefore appears that quindoxin, while a potent photosensitizer is, at most, a weak sensitizer in the absence of light.

DISCUSSION

There is no doubt that quindoxin is capable of inducing photosensitization at very low concentrations. It is perhaps worth stressing that in none of the patients described was the chemical handled as such, the only source of contact being animal feeds to which quindoxin had been added, before purchase, in a concentration of the order of 20–50 mg/kg. As quindoxin is used throughout Great Britain and Ireland and in a number of other countries, it is surprising that all the recorded cases to date have been reported from Northern Ireland. There is no obvious local factor which accounts for restriction of cases to Northern Ireland, and it is therefore likely that further cases will appear in other areas.*

It is clear from the results of the investigation that standard closed patch-tests will lead to a large number of false negative results at 48 h, and that the diagnosis can best be established by carrying out a photo-patch-test. Furthermore, it is suggested that exposure of the test site to sunlight for a few minutes, even when this is filtered through heavy cloud, may be as satisfactory a method of demonstrating this allergy as the use of an ultraviolet light lamp.

ACKNOWLEDGMENTS

We are indebted to Dr C.W.Marsden (Pharmaceuticals Division) and Dr K.S.Williamson (Industrial Hygiene Research Laboratories), Imperial Chemical Industries Ltd, for supplying us with information about quindoxin and sending us a sample of it. We are similarly indebted to Messrs N.M. Ashwell and R.J.H.Williams, Monsanto Chemicals Ltd, in respect of Santoquin. We also wish to thank Miss M.H.Smith, AIIP, Craigavon Area Hospital, for the clinical photograph.

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* Five similar cases have been diagnosed in the Cambridge area (Editor).

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Pharmacology and Treatment

Blood prostaglandin activity associated with noradrenaline-provoked flush in the carcinoid syndrome

A.G.SMITH AND M.W.GREAVES

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology,
Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP

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SUMMARY

A case of the carcinoid syndrome with unusual skin lesions is described. A rise in blood prostaglandin activity associated with noradrenaline-induced flushing is reported. The significance of prostaglandins in the carcinoid syndrome is discussed.

CASE REPORT

A 73-year-old widow was referred with a 2-year history of an itchy rash on the abdomen. Itching preceded the formation of blisters, each of which healed over a period of about 6 weeks to leave a white scar-like lesion. After 1 year the blistering ceased, but the itching persisted. Over the same period, she admitted to attacks of flushing of the face. These occurred between one and six times a day, and each lasted about a minute. She also complained of diarrhoea with up to three semi-formed motions per day. She had noted that flushing coincided with her bowel action and also exacerbated her itching. During the first year of her illness she had lost over 12 kg in weight, but recently there had been an increase in weight due to the development of congestive cardiac failure, with swelling of the legs and abdomen, and increasing breathlessness.

On examination there was gross telangiectasia of the face and neck, both in the form of dilated venules and dot-like petechial angiomata (Fig. 1). A diffuse erythema involved both the dorsa and palms of the hands. On the abdomen there were many white macules each with a halo of erythema and telangiectasia (Figs. 2 and 3).

In addition, she was in congestive cardiac failure with signs of tricuspid valve incompetence and stenosis. The liver was enlarged four fingers' breadth below the right costal margin, and its edge was nodular.

Investigations

The diagnosis of the carcinoid syndrome was confirmed by finding the 24 h urinary excretion of 5-hydroxyindoleacetic acid to be increased to between 200 and 400 mg (normal 4–14 mg). The serum alkaline phosphatase was raised to 116 units (normal 20–90 units), suggesting the presence of hepatic secondary deposits. This was confirmed by a liver scan which showed several filling defects. No radiological evidence of the site of the primary carcinoid tumour was found in either the chest or the intestines.

Flushing in response to intravenous noradrenaline (Robertson, Peart & Andrews, 1962) is a specific and sensitive test for the carcinoid syndrome. The patient was given 2 μg noradrenaline intravenously; after 1 min, flushing of the face occurred, lasting 2 min. This test was repeated about 1 h later with similar results.

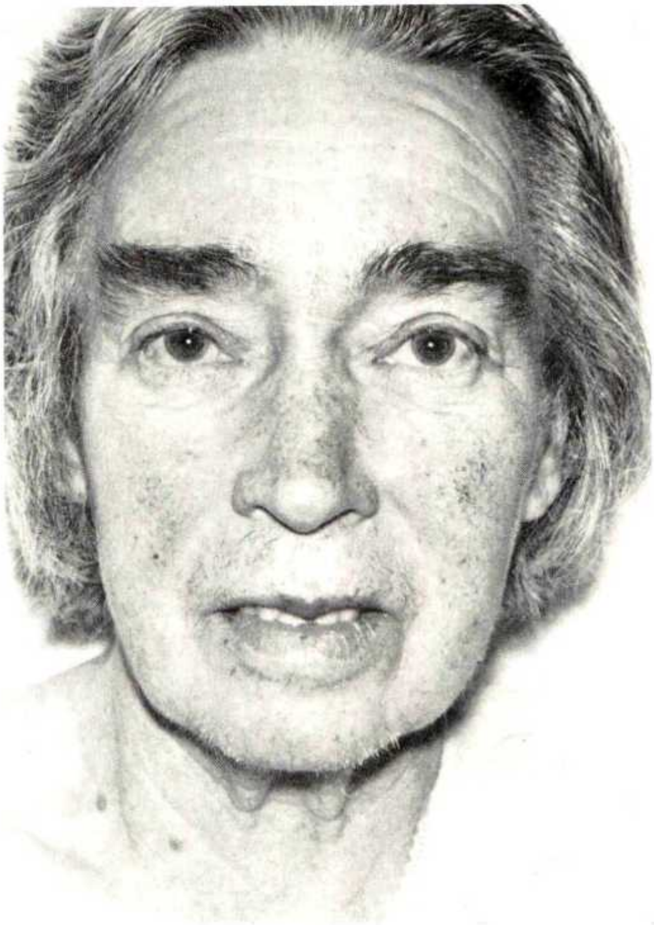

FIGURE 1. Cross facial telangiectasia.

Histological examination of the white macules on the abdomen showed no differences from nearby skin. Both showed epidermal atrophy, dilated dermal vessels and oedema and a scanty lymphocytic infiltrate.

Prostaglandins

Blood samples were taken both before noradrenaline injection and during the subsequent flushing, for estimation of whole blood prostaglandin activity. 10 ml of blood were removed without anticoagulant, from an unoccluded forearm vein both before and immediately after provocation of a flushing attack by intravenous administration of 2 μg noradrenaline. Whole blood prostaglandin

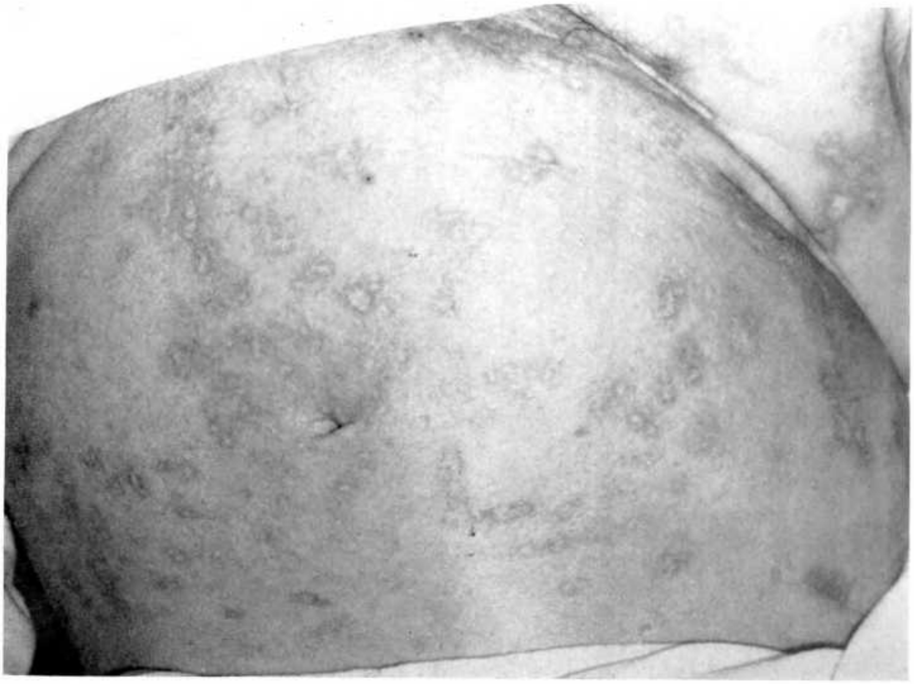

FIGURE 2. White macules on the abdomen, each surrounded by a halo of erythema and telangiectasia.

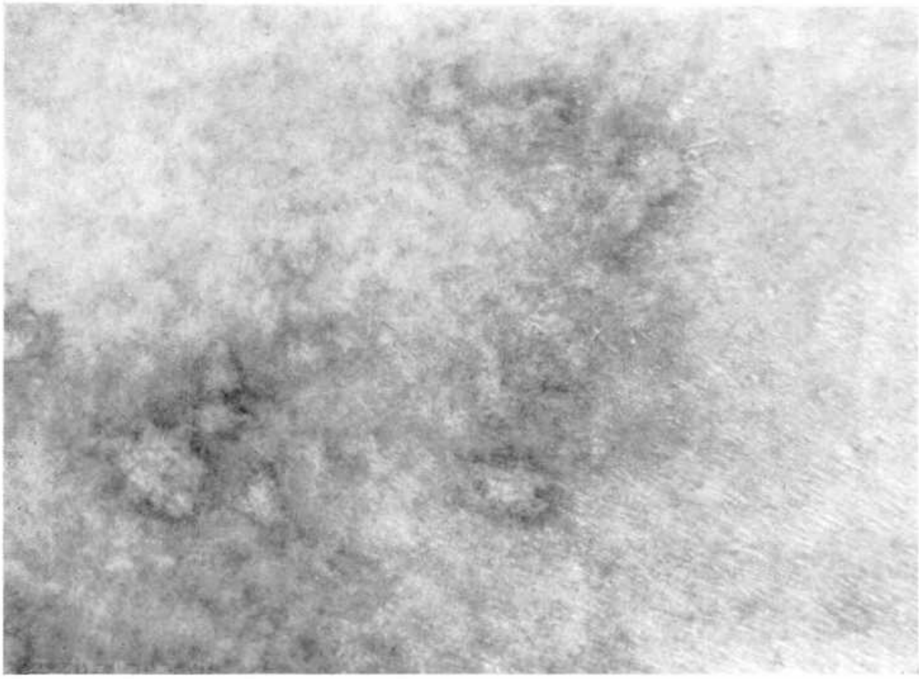

FIGURE 3. Close-up of Fig. 2.

activity was determined by the method of Greaves & McDonald-Gibson (1972). Acidic lipids were extracted from haemolysed blood using ethanol, petroleum ether and diethyl ether, followed by thin layer chromatography. Prostaglandin activity in eluates from silica gel and from final ether extracts was determined by bioassay using the isolated rat uterus preparation.

Before injection of noradrenaline, the total whole blood prostaglandin activity was 0·35 ng/ml, rising to 2·0 ng/ml after noradrenaline. Thin layer chromatography showed that, of the total prostaglandin activity recovered before noradrenaline, approximately two-thirds was prostaglandin E and the remainder was prostaglandin F. After noradrenaline the recovered prostaglandin activity was due almost entirely to prostaglandin E.

DISCUSSION

The significance of different vasoactive substances found in the carcinoid syndrome is uncertain. Serotonin is not by itself the cause of the carcinoid flush, because the flush cannot be reproduced by intravenous injection of serotonin (Levine & Sjoerdsma, 1963). Histamine is probably important only in certain carcinoid tumours arising from the foregut (Oates & Sjoerdsma, 1962). These patients have a characteristic bright red blotchy flush. Bradykinin produces flushing, and kinin activity has been found in high concentrations in hepatic venous blood of patients with liver metastases in the carcinoid syndrome (Oates *et al.*, 1964). More recently, increased blood kinin activity has been detected during carcinoid flushing (Zeitlin & Smith, 1966; Adamson *et al.*, 1969), and it seems possible that kinins contribute significantly to the carcinoid flush.

Administration of prostaglandin E_1 or E_2 in man also causes intense flushing, associated with diarrhoea (Bergstrom *et al.*, 1959, 1965; Horton *et al.*, 1968). The presence of prostaglandin in carcinoid tumour tissue has recently been reported by Sandler, Karim & Williams (1968), and these authors also found a prostaglandin-like hydroxy fatty acid in peripheral blood from two patients with mid-gut carcinoid tumours. The present patient is the first in whom a rise in whole blood prostaglandin activity has been demonstrated in association with the carcinoid flush. That the absolute values both before and during flushing are well within normal limits (Greaves & McDonald-Gibson, 1972) is not surprising in view of the presence of large concentrations of prostaglandin-degrading enzymes in the lungs and blood (Ferreira & Vane, 1967; McDonald-Gibson, McDonald-Gibson & Greaves, 1972). The significance of the noradrenaline-evoked rise in blood prostaglandin activity is speculative. A direct effect of noradrenaline on prostaglandin biosynthesis seems unlikely: expulsion of a pool of prostaglandin-rich blood from the hepatic vasculature into the systemic circulation in response to the vasoconstrictive action of noradrenaline seems more probable. However, the possibility that increased prostaglandin activity after noradrenaline is independent of the carcinoid tumour cannot be excluded, since noradrenaline has a well-known α-adrenergic fat mobilizing effect which leads to formation and release of prostaglandins from adipose tissue (Shaw, 1966). Confirmation of increased prostaglandin activity in the carcinoid syndrome will probably have to await an opportunity for direct sampling of hepatic venous blood. If confirmed, the observation of increased prostaglandin activity in the carcinoid syndrome may be of significance, since apart from mediating flushing and diarrhoea, prostaglandins may play a role in the production of the endocardial fibrosis affecting the right side of the heart, and the rarer fibrosis involving other sites; namely peritoneal fibrosis, lung adhesions, constrictive pericarditis, and retroperitoneal fibrosis (Grahame-Smith, 1972). Both prostaglandin E and prostaglandin F are known to cause increased collagen biosynthesis (Blumenkrantz & Sondergaard, 1974), and anti-inflammatory drugs which inhibit prostaglandin biosynthesis are known to inhibit collagen synthesis (Nakagawa & Bentley, 1971; Whitehouse & Bostrom, 1965). Thus anti-inflammatory drugs which inhibit prostaglandin biosynthesis (Greaves & McDonald-Gibson, 1973) may be of value in the treatment of acute and chronic manifestations of the carcinoid syndrome.

Itching is rare, but is described in the carcinoid syndrome (Mengel, 1963). Blistering, as described by the patient and substantiated by her family, does not appear to have been described before. However, Bean & Fusaro (1968) described a patient with the carcinoid syndrome, who had a number of erythematous, telangiectatic atrophic plaques with central ulceration. The blisters and observed lesions in our patient may represent similar lesions at different stages in their evolution. Both the blisters and lesions reported by Bean probably result from scratching and owe their final morphology to the vasoactive substances secreted by the tumour.

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Biological comparison of isomers and chemical forms of vitamin A (retinol)

R.I.C.SPEARMAN AND A.JARRETT

Dermatology Department, University College Hospital Medical School, London, W.C.1

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SUMMARY

The effects were compared on mouse tail scale epidermis of vitamin A acid, acetate and alcohol (all as the *trans* isomer) and the *cis* aldehyde by regular topical applications. Increases in epidermal thickness produced by different doses were compared statistically, and the induction of a granular layer and of epidermal alkaline phosphatase activity was demonstrated.

The different forms and isomers of vitamin A have similar effects on the epidermis, but vary individually in activity. Retinoic acid and *trans*-retinyl acetate were very similar in their action when used in a water solubilizing vehicle. The fact that retinyl acetate is very much cheaper than retinoic acid is a point in favour of its use for the treatment of psoriasis.

It has become increasingly evident, since the first reports of the value of vitamin A in psoriasis (Jarrett & Spearman, 1959), that retinyl acetate has a beneficial effect on parakeratotic keratinization in man. However, the results are not uniformly good because the vitamin labilizes lysosomes and in consequence may produce adverse irritant effects. Because of this, it was thought advisable to combine the vitamin with triamcinolone to reduce the irritant effect and the consequent mitotic stimulation. This combined application was found to improve a larger range of psoriatics than either did alone (Jarrett & Spearman, 1959; Whittle *et al.*, 1961; Jarrett & Spearman, 1964; Riley, 1969; Jarrett & Spearman, 1970; Jarrett, 1973).

More recently, retinoic acid has been reported as being of value for the treatment of psoriasis (Frost & Weinstein, 1969), and even more recently the combination of this with fluorinated steroids has been evaluated (Orfanos *et al.*, 1973). It has been suggested by some that retinoic acid has properties differing from those of other forms of vitamin A and that its use is therefore an advance in this mode of therapy. Nevertheless, in experimental animals it has been found to supply all metabolic needs for vitamin A, except for the regeneration of rhodopsin in the retina (Roels, 1967). Retinoic acid is many times more expensive than retinyl acetate, and it was therefore thought worthwhile to evaluate the effects on mouse tail epidermis of a number of different chemical types and isomers of vitamin A. Montagna (1954) demonstrated poor penetration of guinea-pig epidermis by oily preparations of vitamin A, and we found that retinyl acetate does not readily penetrate directly through either human or animal epidermis, unless it is made artificially water-soluble (Jarrett & Spearman, 1959, 1964). We therefore decided to compare different kinds of vitamin A made artificially water-soluble by a special water-solubilizing solution, kindly supplied by Beechams Research Laboratories. This solvent, which contains an antioxidant and tweens, renders the vitamin water-soluble and more able to pass directly through the epidermis. Penetration can be established because of the specific action of vitamin A in inducing alkaline phosphatase activity in the lower layers of the mouse epidermis; this induction does not occur in human skin (Jarrett & Spearman, 1964). Moreover, it has been shown by Riley & Spearman (1968) that retinyl acetate induces the production of an epidermal iso-enzyme of alkaline phosphatase that is different from the alkaline phosphatase normally present in the dermal vessels and the granular layer.

MATERIALS AND METHODS

The animals used in these experiments were adult male albino mice of the 'to' strain: each weighed about 30 g. The forms of vitamin A investigated were retinyl acetate, retinoic acid, and the alcohol (Retinol) (all as the *trans*-isomer), and *cis*-retinol aldehyde. They were made up in a number of concentrations varying from 1000 to 30,000 units/ml of solvent (when making up the solutions, 0·0003 mg was taken as equivalent to one international unit of vitamin A). In the case of vitamin A acetate, concentrations exceeding 30,000 units were used, as we knew from previous experience that these large doses were not highly irritant. Each concentration was painted once daily, with a camel hair brush, onto mouse tails, for a total period of 3 weeks. The number of animals used for each concentration varied from four to ten (see Tables); the maximum number of animals in a cage was limited to five.

At the end of the experimental period, the animals were killed and their tail skin removed. A rectangular piece of skin situated on the dorsum, 2 cm from the base of the tail, was fixed in 70% ethanol and processed for subsequent histological examination. Measurements of the tail epidermal thickness were made from the dermo-epidermal junction to the base of the horny layer. Ten measurements were made for each of ten scale regions in each mouse. The thickness of the mouse tail scale epidermis was then calculated as the arithmetic mean of these 100 readings. This technique was used in order to be uniform and comparable with the results already published on vitamin A acetate in previous experiments (Jarrett & Spearman, 1964).

By this means, values for the thickness of the tail epidermis were obtained for each of the concentrations of the various kinds of vitamin A (see Tables). From these, the dose curve response for each could be calculated, and, with the aid of a computer, values for regression coefficients for each type were determined. These could then be compared, and their relative effects on mouse tail epidermis assessed.

The presence or absence of a granular layer, and the appearance of the epidermis and horny layer, were observed in paraffin processed sections fixed in 70% ethanol and stained with haematoxylin and eosin. Keratohyalin granules are most clearly demonstrated after ethanol fixation (Jarrett & Hardy, 1957). Sections were also examined for any pathological changes induced in the epidermis or dermis by the different forms of vitamin A.

Alkaline phosphatase was demonstrated in paraffin sections by the azo dye coupling method, using fast red TR and α-naphthyl phosphate buffered to pH 9·2 (Bancroft, 1967).

RESULTS

Metric data

The thickness of the tail epidermis, following the application of the various types of vitamin A and their different concentrations, is given in Table 1. Table 2 shows the values with the solvent alone and with a group of untreated mice of the same strain.

It will be seen that in general there is an increasing thickness of tail epidermis with increasing concentrations. The one marked exception is the 15,000 unit/ml concentration of the *trans*-retinol. The most likely explanation for this apparently anomalous result is that there was a great amount of epidermal damage, and this probably interfered with the mitotic activity of the epidermal cells: also the development of intra-epidermal blisters tended to make measurements inaccurate.

TABLE 1. Average thickness of tail scale epidermis for each animal group

Dose of vitamin (units/ml)	Log dose of vitamin	*trans*-retinyl acetate (μm)	*trans*-retinoic acid (μm)	*trans*-retinol (μm)	*cis*-retinol aldehyde (μm)
1000	3·0000	39·29 (10)*	48·60 (5)	44·73 (5)	45·49 (5)
5000	3·6990	57·64 (10)	56·51 (5)	48·61 (5)	61·25 (5)
7500	3·8751	—	58·90 (8)	45·04 (5)	71·10 (5)
10,000	4·0000	—	50·66 (5)	66·37 (5)	—
12,500	4·0969	60·09 (9)	—	—	—
15,000	4·1761	—	69·23 (10)	48·74 (4)	—
25,000	4·3979	69·10 (6)	—	—	—
30,000	4·4771	—	70·90 (7)	65·32 (4)	66·82 (5)
50,000	4·6990	77·60 (6)	—	—	—
100,000	5·0000	76·48 (5)	—	—	—

*Number of animals in each group.

TABLE 2.

	No. of animals	Average thickness of scale tail epidermis (μm)
Solvent alone	10	40·98
Control untreated animals	11	26·1

The relationship between epidermal thickness and the $\log_{10}$ concentration of the vitamin isomers appeared to be approximately linear, and straight lines were fitted by the method of least squares. The values for the four forms of vitamin A are shown in Table 3, together with measurements of the variation about the lines, a small variation indicating that the values fit the line closely. This was especially so with *trans*-retinyl acetate, where the values were very close to the line, and a high degree of confidence can be placed in the value for the slope of the line (Fig. 1). The variation for the *trans*-retinol is rather large, and no great confidence can be placed on the value of the slope, which is shown by a dotted line in Fig. 1.

The values of the slope of the line indicate the rate of increasing thickness for each tenfold increase in the concentration of the vitamin. Thus, the theoretical difference in thickness between 1000 units of *trans*-retinyl acetate and 100,000 units should be 19·796 × 2 (i.e. 100-fold increase = 39·592). The observed difference (column 3, Table 1) is 76·48 (value for 100,000 units) −39·29 (value for 1000 units) = 37·19.

TABLE 3.

	Slope of line	Variation about line
trans-retinyl acetate	19·796	Stable
cis-retinol aldehyde	15·971	3·46
trans-retinol	12·620	5·82*
trans-retinoic acid	16·112	2·76

* Too large for a high degree of confidence in the value for the slope of the line.

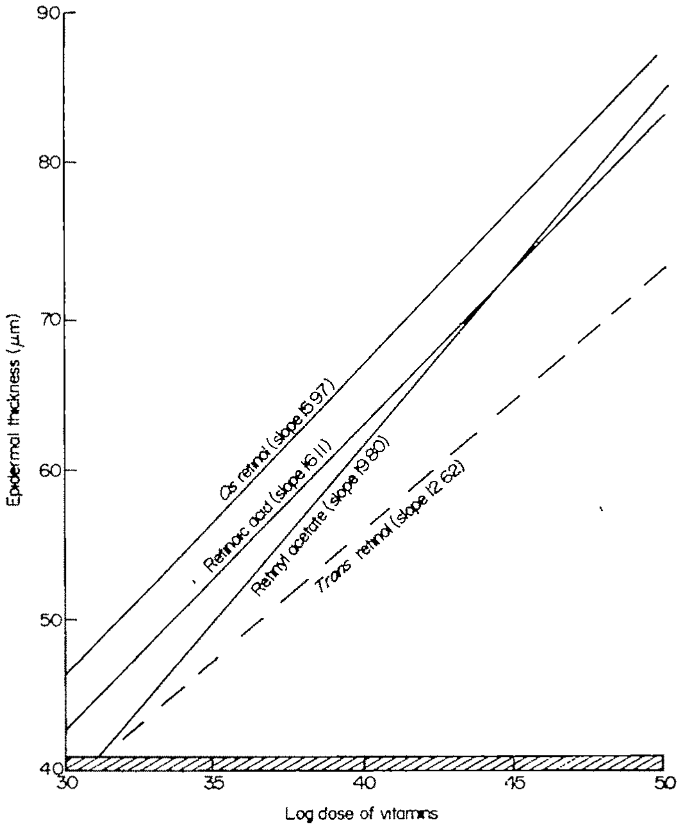

FIGURE 1. Log. dose slopes for different forms of vitamin A after topical application to mouse tail skin for 3 weeks, showing thickening effect on tail scale epidermis at different concentrations. *cis*-retinol produced the same degree of thickening as double the dose of retinoic acid at all concentrations. Retinyl acetate had less thickening effect than retinoic acid at low concentrations. The line for *trans*-retinol is shown interrupted, as variation in thicknesses exceeded reasonable confidence limits. This variation was probably due to the greater damage to the epidermis produced by *trans*-retinol. Hatched area represents thickening due to solvent alone.

Histological and histochemical changes

Induction of granular layer. The untreated tail epidermis has a granular layer in the follicular regions, but this layer is absent in the scales. The undiluted solvent failed to induce a granular layer in the tail scales, although it had a slight thickening effect on the epidermis. All forms of vitamin A at a concentration of 30,000 units/ml induced a granular layer several cells in depth in the tail scale epidermis. At lower concentrations, *cis*-retinol aldehyde and *trans*-retinol induced a patchy granular layer. Both retinyl acetate and retinoic acid were potent inducers of granular layer formation with a patchy distribution at 5000 units/ml and one several cells in depth at 7000 and 10000 units/ml (Fig. 2).

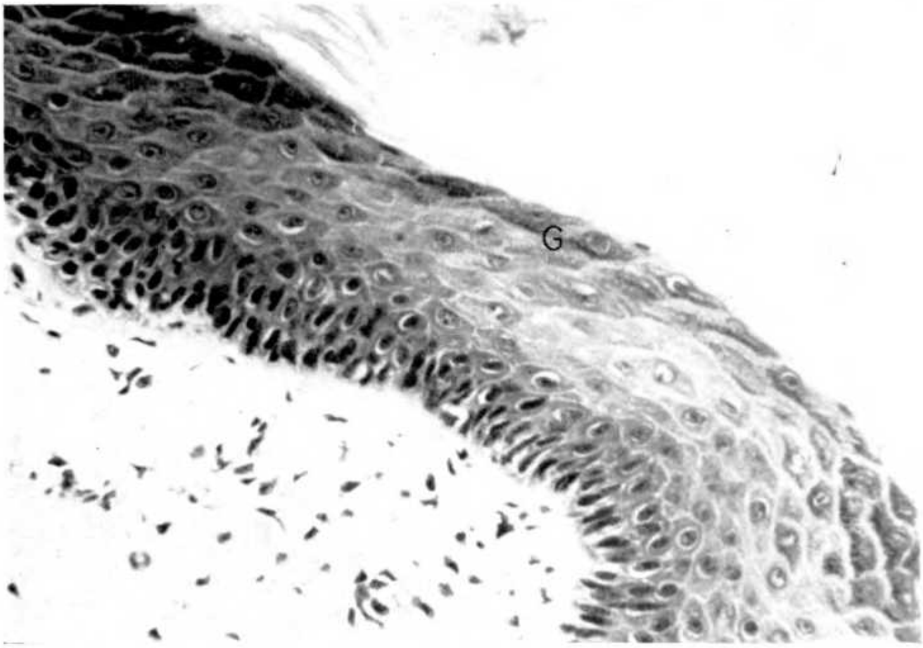

FIGURE 2. Mouse tail epidermis after topical retinoic acid (7500 units/ml) for 3 weeks, showing induction of granular layer (G) in the scale region (H & E, × 448).

Horny layer desquamation. Normal mouse tail scale horny cells are compacted together and are occasionally shed in large fragments. After treatment with all forms of vitamin A there was a tendency for the scale horny layer to separate into several thin sheets.

Alkaline phosphatase. Induction of alkaline phosphatase activity in the lower half of the tail scale epidermis occurred with all the types of vitamin A (Fig. 3), but the solvent alone did not induce this enzyme.

At all concentrations there was enlargement of prickle cells which had prominent nucleoli. Basal cells had small compact, darkly stained nuclei.

Damage to the epidermis. None of the forms of vitamin A examined damaged the epidermis at concentrations of 5000 units/ml or below. All caused some intercellular oedema at 7000 units/ml. At higher concentrations, most damage was caused by *trans*-retinol, and least damage by *cis*-retinol aldehyde, with intermediate but similar effects of retinoic acid and retinyl acetate. The most severe damage was seen as local cell necrosis leading to the formation of intra-epidermal bullae, particularly

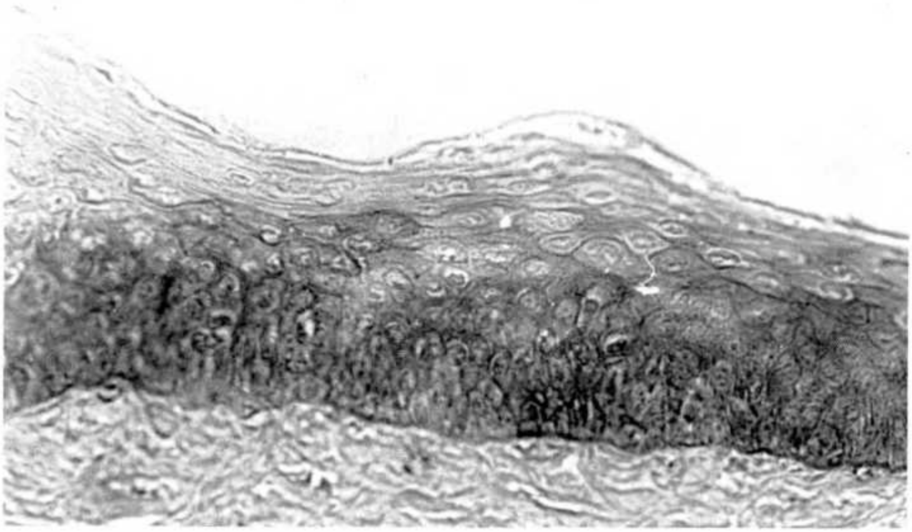

FIGURE 3. Mouse tail skin after topical retinoic acid (7500 units/ml) for 3 weeks, showing induction of alkaline phosphatase in lower half of epidermis (α-naphthyl phosphate and fast red TR, ×448).

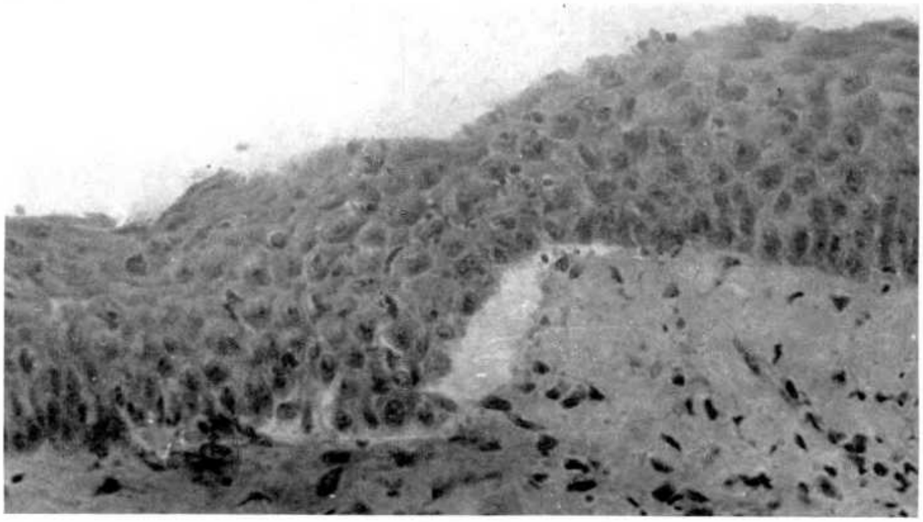

FIGURE 4. Mouse tail skin after topical retinoic acid (7500 units/ml), showing damage to the dermo-epidermal junction and spongiosis in prickle cell layer (H & E, ×448).

in the region just beneath the granular layer, and occasionally subepidermal bullae were seen (Fig. 4). Where this occurred, accurate measurements of epidermal thickness were difficult to obtain.

DISCUSSION

It has been shown that the two isomers and various chemical forms of vitamin A all have similar effects on mouse tail epidermis, although these vary in degree. There is an increase in epidermal thickness, which is a combination of increased cellular proliferation (Laurence & Bern, 1958; Sherman, 1961), and increased prickle cell volume (Montagna, 1954). There is also an associated stimulation of RNA synthesis (Zachman, 1967; Lukács, Christophers & Braun-Falco, 1971). Induced formation of a new iso-enzyme of alkaline phosphatase by mouse epidermal cells is stimulated by retinyl acetate (Riley & Spearman, 1968), and similar distributions of this enzyme induction after applications of other forms of vitamin A suggest that all act in the same manner on mouse epidermis. Formation of alkaline phosphatase in the scale epidermis indicates that the vitamin penetrates directly through the keratin layer.

Measurements of horny layer thickness are unsatisfactory because of desquamation. However, Braun-Falco & Christophers (1969) found that it was thinned by topical retinoic acid. In the present investigation (peeling) desquamation of the normally compact horny scales in thinner fragments occurred at doses of 5000 units/ml and above, on all forms of vitamin A treatment. This desquamation is probably associated with an alteration of epidermal keratinization, as previously demonstrated for retinyl acetate (Jarrett & Spearman, 1964).

Induction of a granular layer in the tail scale epidermis, which normally does not have such a zone, is a characteristic and specific effect of all forms of vitamin A so far tested. Hairy sites, after topical treatment with vitamin A, show a general thickening of the epidermis, including the granular layer which becomes several cells in depth (Sabella, Bern & Kahn, 1951; Laurence & Bern, 1958; Montagna, 1954). However, to demonstrate a specific effect, the actual induction of keratohyalin granule formation must be shown to occur at a site where this material is not normally present (Jarrett & Spearman, 1964). In this respect, tail skin is a useful test site because the induction of a granular layer with lower vitamin concentrations is associated with a change in keratinization towards that of normal human epidermis: an indication that such treatment might improve psoriatic lesions. Formation of keratohyalin occurs also in the parakeratotic lesions of psoriasis treated with topical vitamin A as was first shown by Puccinelli & Bellone (1948). A change to granular layer formation would be expected to be accompanied by a return to normal keratinization in psoriatic lesions, but this is complicated by the adverse stimulatory effect of vitamin A on epidermal proliferation (Reid & Jarrett, 1967). Probably for this reason, the lesions in the patients of Puccinelli & Bellone failed to improve, and consequently their investigation was discontinued. Indeed, the stimulatory action of the higher concentrations of the various forms of vitamin A is likely to produce exacerbation of psoriatic lesions. It is for this reason that treatment with vitamin A alone was usually unsatisfactory, and that its combination with fluorinated corticosteroids was suggested (Jarrett & Spearman, 1964; Orfanos *et al.*, 1973).

The log. dose curves for the epidermal thickening effect of vitamin A are clearly unrelated to ability to induce a granular layer with a consequent change in keratinization. At low concentrations, retinyl acetate had less thickening effect than retinoic acid, but they were equally effective in granular layer formation. This would suggest that retinyl acetate is of more value for the treatment of psoriasis. The thickening action of *cis*-retinol aldehyde paralleled that of retinoic acid (Fig. 1). As this is a log. dose graph it indicates that the *cis*-retinol produces the same degree of thickening as double the dose of retinoic acid. However, despite the greater thickening effect of *cis*-retinol aldehyde, it was a poor inducer of a granular layer. In psoriasis an epidermal thickening effect is undesirable, and therefore retinoic acid is to be preferred to the *cis*-retinol for topical therapy.

With respect to granular layer induction, retinyl acetate and retinoic acid are very similar, but the *cis*-retinol aldehyde was much less active. *Trans*-retinol caused most epidermal damage at higher concentrations, and this is probably the reason for the great variations in thicknesses of tail epidermis that occurred in this series.

The relative oil/water solubility of the different forms of vitamin A have not been compared, but differences in this respect will affect epidermal penetration. Thus, Sobel *et al.* (1958) found that water solubilization of retinyl acetate by means of tweens significantly increased its percutaneous absorbtion in rats, as measured by serum and liver levels of vitamin A. Laurence & Bern (1958) achieved good results with water solubilized vitamin A on mouse epidermis. We found that it was essential for the retinyl acetate to be made water soluble for it to be effective in mouse and human skin (Jarrett & Spearman, 1964). Retinoic acid appears to be more soluble in water and is effective without a water solubilizing vehicle. This probably accounts for its popularity as a local therapeutic agent. However, its cost compared with retinyl acetate is great, and the penetration of the acetate can readily be made equal to that of the acid by the use of special solvents, so that there would seem little point in employing the expensive form of vitamin A.

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Successful surgical treatment of lipoid proteinosis

N.G.BUCHAN AND J.V.HARVEY KEMBLE

Department of Plastic Surgery, Queen Mary's Hospital, Roehampton, London, S.W.15

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SUMMARY

The facial lesions of a boy with primary lipoid proteinosis were successfully treated by dermabrasion, chemical skin peeling, and blepharoplasty.

CASE REPORT

A boy aged 16 was first seen at the age of 15 months by Dr E.J.Moynahan, because of blistering lesions on the face and scalp. These blisters healed with residual ice-pick scars over all his face. He also had keratotic plaques on the fingers, elbows and toes. A diagnosis of lipoid proteinosis was made at this time. He had had hoarseness since his first attempts to make sounds. There was no history of parental consanguinity or of epilepsy. His four siblings are normal.

At the age of 9 he was given prednisolone orally for 2 months but right and left ventricular hypertrophy developed and the drug was discontinued. Erythromycin 250 mg three times daily, by mouth, and Terra-Cortril spray topically were used for occasional septic blisters on the face.

Two years ago he was referred to Mr A.J.Evans for advice regarding plastic surgery to the face. At that time he was a thin, pale youth whose voice was markedly hoarse. Small papules covered his face which was waxy and firm to the touch (Figs. 1 and 2); his lower eyelids were beaded and excessively wrinkled (Fig. 3). He had hyperkeratotic plaques on the elbows, the palms and the volar aspects of the fingers. The oral cavity and tongue were normal. An apical systolic cardiac murmur was not considered significant.

Investigations (see Table 1)

Methods of treatment: (a) Dermabrasion (Wynn-Williams, 1959). A silicone carbide cylinder, driven by a high torque motor (Kavo-Kaltenbuch) to speeds up to 12,000 rev/min, was used to abrade a small area of skin on the right temple, in January 1971. Abrasion of this test area was carried down to a level of moderate punctate bleeding; the raw surface was covered with tulle-gras which peeled off during the following 2 weeks revealing smooth, pink, new epithelium. The erythema faded over 3 months; his forehead, cheeks, lower lip and chin were then dermabraded with similar results.

(*b*) *Chemical skin peeling* (Litton, 1962). This method of applying sparingly a solution of 80% phenol in water to produce a superficial chemical burn, was used on his lower eyelids, nose and upper lip in September 1971. The treated areas healed with the formation of smooth, shiny pink skin which gradually became paler over the following 3–6 months. These areas are difficult to dermabrade because

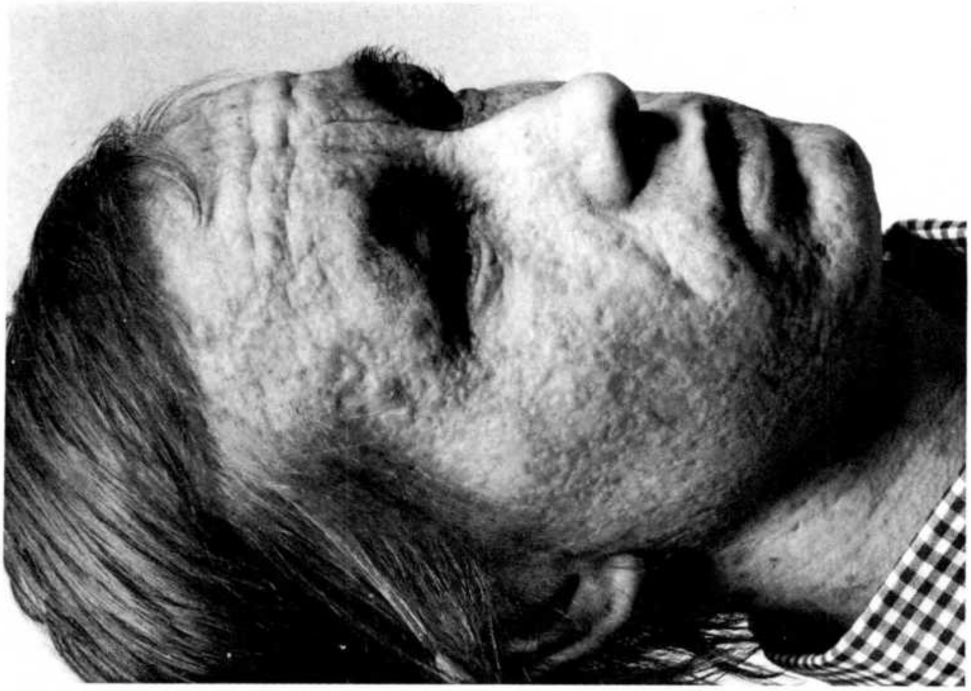

FIGURE 1. Patient at age 14, before any surgical treatment.

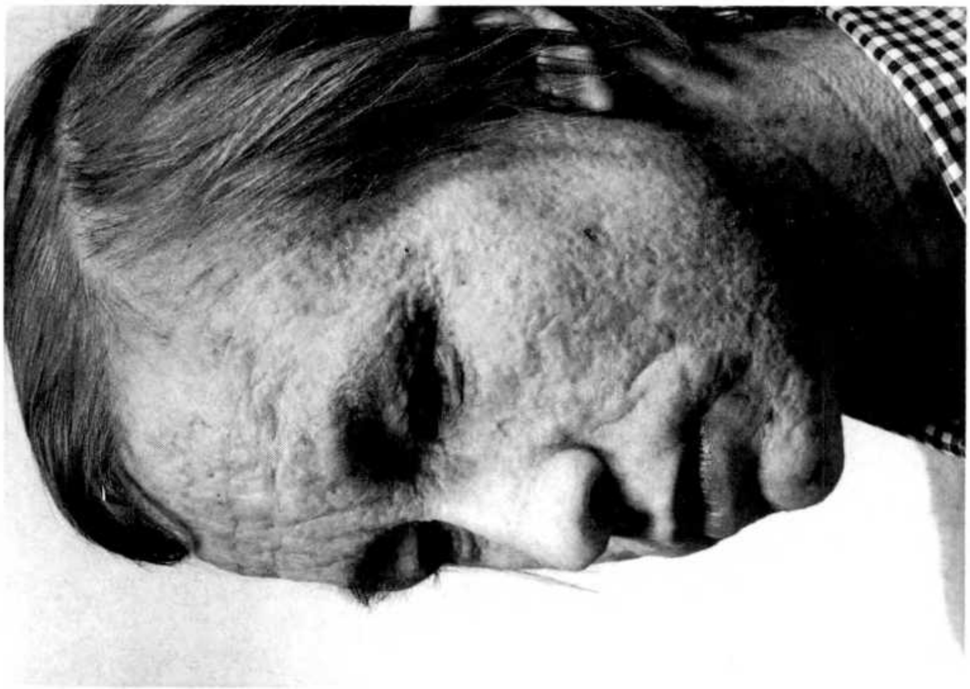

FIGURE 2. Patient at age 14, before any surgical treatment.

of irregular contours or undue laxity of the skin. The appearance of the face, 18 months after the initial surgery, is shown in Figs. 4 and 5.

(*c*) *Blepharoplasty*. The wrinkled lower eyelids were reduced surgically at this time and the result, 1 week after operation, is seen in Fig. 6. Two small, unhealed areas on the left lower eyelid healed subsequently in 1 week.

Histology

This confirmed the clinical diagnosis of lipoid proteinosis (Table 2).

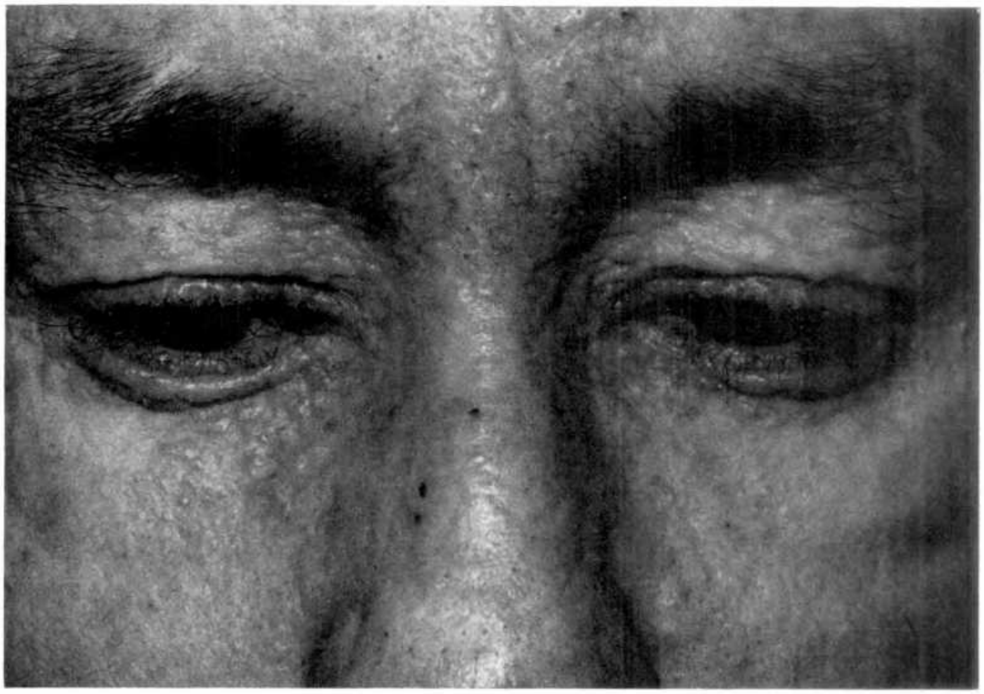

FIGURE 3. Close-up of the front of the middle third of the face, showing the wrinkled, redundant skin of the lower eyelids before blepharoplasty.

TABLE 1. Investigations.

Date	Normal	Date	Abnormal
1971	Hb, WBC SGOT, SGPT and LFTs	1969	ECG: RV and LV hypertrophy
	Serum cholesterol and glucose Plasma proteins ECG Urinary porphyrins and mucopolysaccharides X-ray skull	1971	Plasma electrophoresis—marked increase in globulin fraction

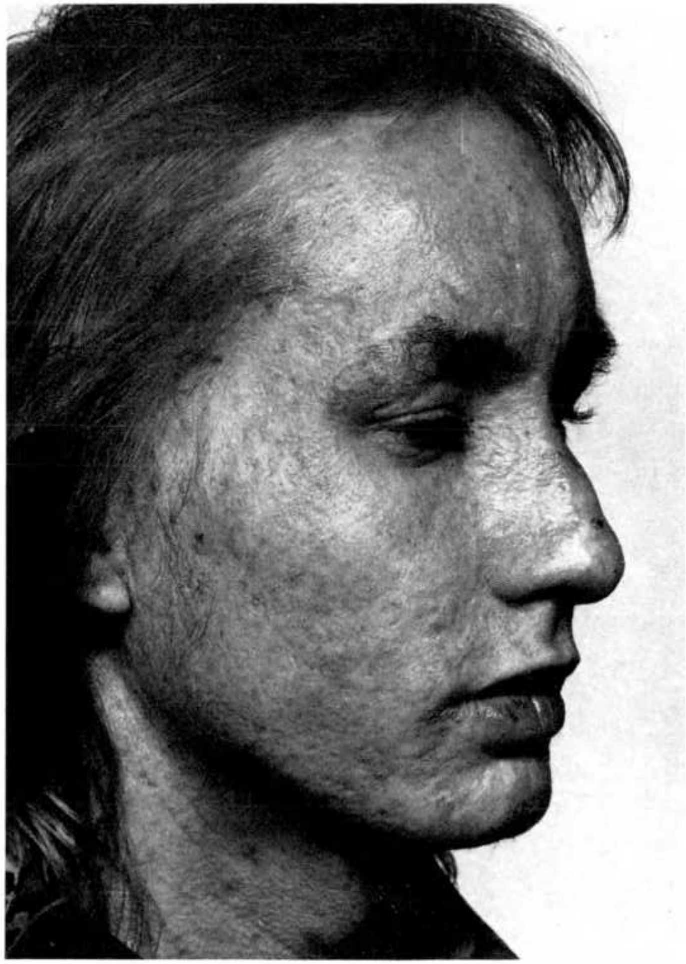

FIGURE 4. 18 months after surgical treatment was begun and before blepharoplasty.

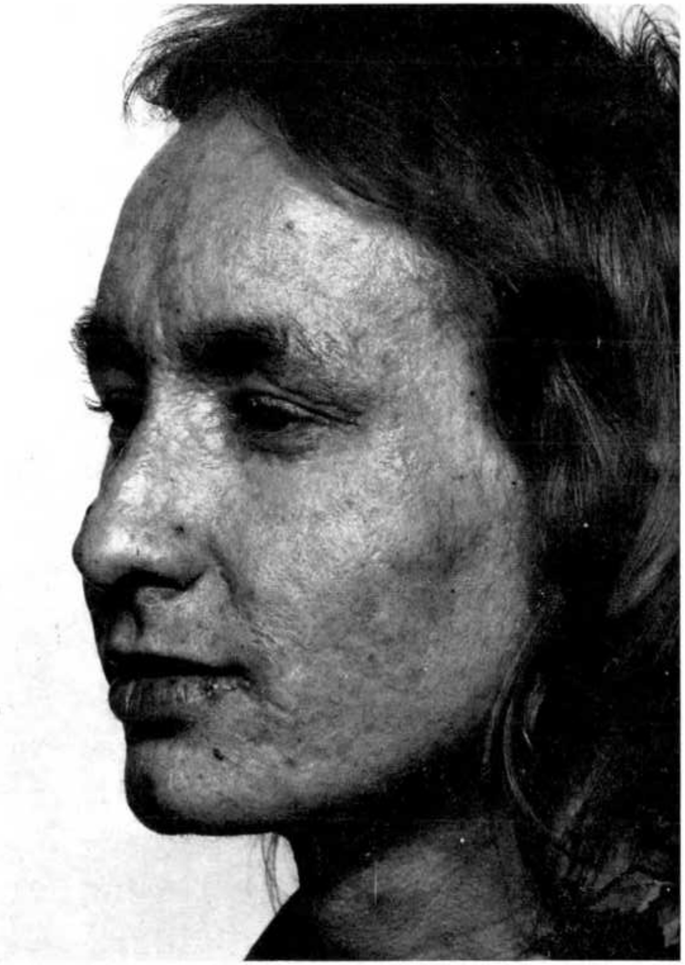

FIGURE 5. 18 months after surgical treatment was begun and before blepharoplasty.

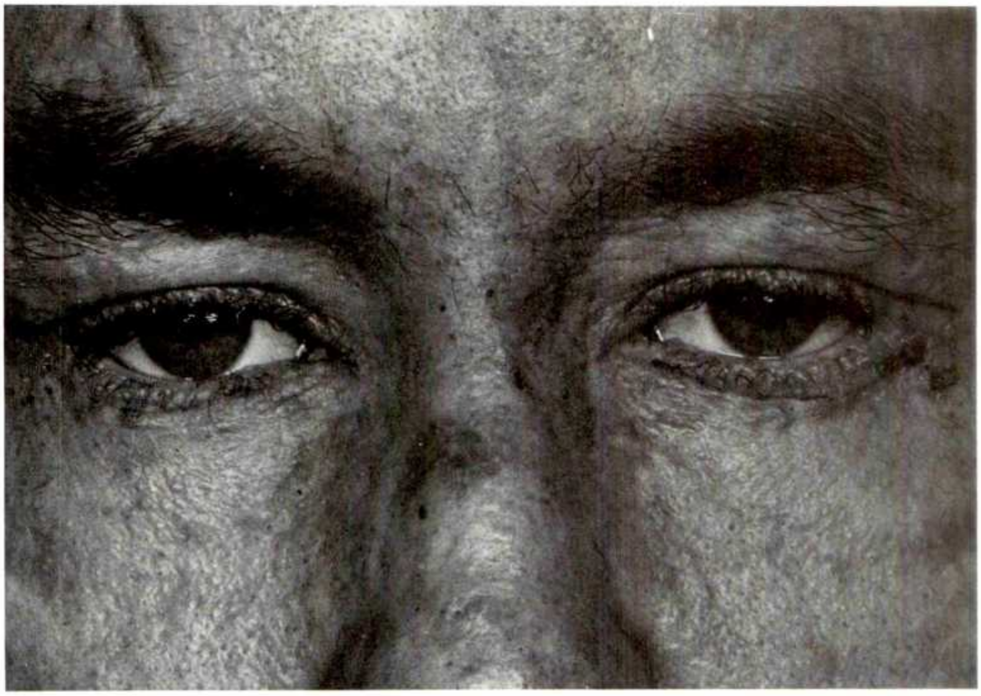

FIGURE 6. Frontal close-up of the middle third of the face showing the result 1 week after blepharoplasty.

TABLE 2. Histology.

Date	Specimen	Stain	Reaction	Significance
1964	Skin from unknown site	Haematoxylin and eosin	No abnormality	Specimen may have been from relatively unaffected site
1964	Vocal cord biopsy	Haematoxylin and eosin	Focal collections of eosinophilic hyaline material in upper dermis and around dermal blood vessels	Corresponds with other authors describing lipoid proteinosis, e.g. Scott & Findlay (1960)
1972	Skin from lower eyelids	Haematoxylin and eosin	Same reaction as in vocal cord biopsy	
1972	Skin from lower eyelids	Periodic-acid Schiff	Positive reaction in hyaline areas; diastase resistant	Suggests lipoprotein or mucopolysaccharide
1972	Skin from lower eyelids	Van Gieson	Greyish-yellow	None
1972	Skin from lower eyelids	Congo Red Sirious Red Crystal violet	No reaction	Not amyloid
1972	Skin from lower eyelids	Oil Red O Sudan III	No reaction	Not lipid

DISCUSSION

No effective therapy has been found for this disease since Siebenmann described a case under the name 'Ichthyosiform Disease' in 1908. Although it is not a threat to life, except, rarely, from laryngeal obstruction (Montgomery & Havens, 1939), it causes considerable anxiety about social acceptability.

Arsenicals have been applied to the skin without benefit (Scott & Findlay, 1960). Success has been claimed with oral steroids (Erberthartinger & Niebauer, 1959; Cowan *et al.*, 1961), when these have been started early and used for long periods. Chloroquine phosphate, given orally, has been claimed to improve the hoarseness (Scott & Findlay, 1960).

Diets have been tried; Montgomery & Havens (1939) found that a diet free from animal fat was ineffective. A totally fat-free diet was claimed to produce a temporary subjective improvement (Scott & Findlay, 1960). Pancreatic extract (Lipocaic) was reported to benefit facial plaques in one of two patients (Tomkins & Weinstein, 1954), but Ross (1957), Raymond-Jones (1965) and Cairns (1968) reported failure of this treatment.

According to Wynn-Williams (1959), dermabrasion was first used by the ancient Egyptians to treat scars; Kromayer, in 1905, mechanized the method. Its use in lipoid proteinosis has not, to our knowledge, been described. The merit of this method is that systemic therapy, with possible side effects, is avoided. Milia and altered pigmentation may occasionally occur after dermabrasion. The development of these changes is not predictable. It is therefore advisable to test a small area in an inconspicuous site. A patch of light pigmentation occurred on the right temple of our patient but faded completely over 3 months. Systemic ill-effects from topical phenol have been noted only when more than 3 ml has been used (Litton, 1962). Care should be taken to avoid applying phenol to previously dermabraded areas as excessive scarring may result. Reduction of redundant lower eyelid skin is a well tried technique. Caution should, however, be exercised with eyelids treated by phenol application as ectropion may easily occur in inexperienced hands.

The improved appearance and morale of our patient leads us to recommend further use of these methods of treatment.

ACKNOWLEDGMENTS

The authors wish to thank Mr A.J.Evans, under whose care the patient was treated, and Dr P.W.M. Copeman for their helpful advice and criticism. They also wish to thank Dr J.H.O.Earle for the histology sections and Mr E.Ferrill for the photographic illustrations.

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Case Reports

Pustular psoriasis in association with renal amyloidosis

RONA M. MACKIE AND JOHN BURTON

University Department of Dermatology, Western Infirmary, Glasgow, and Renal Unit, University Department of Medicine, Royal Infirmary, Glasgow

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SUMMARY

A case of chronic pustular psoriasis is reported, with histological evidence of renal amyloid deposition.

There are few reports in the literature of amyloidosis complicating the chronic dermatoses. Although psoriasis is a common dermatological problem, associated amyloidosis has been described in only five cases. We report a sixth case in which the diagnosis of renal amyloid has been made relatively early in the disease process.

CASE REPORT

M.K., a female now aged 44 years, first developed skin lesions at the age of 18. These were areas of axillary erythema with superficial pustules which ruptured rapidly on friction. There was no family history of psoriasis or other dermatological disorder. Her general health was good and she sought no medical advice at this time. The lesions persisted for 4 years and then cleared spontaneously. For the next 5 years the patient was free of skin lesions and during this period she had an uneventful pregnancy.

During her second pregnancy, the axillary lesions reappeared, and involvement of the groins and upper thighs became a feature. Dermatological advice was sought and in-patient treatment required. The diagnosis of impetigo herpetiformis was considered at this time, but in view of the past history and of the persistence of the lesions after delivery of a normal infant, the diagnosis was modified to pustular psoriasis. No biopsy was carried out at this time.

Over the past 17 years, the body flexures have been almost constantly moist and erythematous (Fig. 1). The patient has had only brief periods of freedom from these lesions, and on several occasions there has been involvement of large areas of the trunk and limbs with superficial sterile pustules. Five lengthy periods of in-patient therapy have been required in the past 7 years, and seven skin biopsies have been taken in this period. The biopsies have consistently shown subcorneal pustules, thickening of the rete ridges, and scattered areas of parakeratosis and acanthosis (Fig. 2). Staining of sections with the Gomori method for alkaline phosphatase has shown the tortuous dilated capillaries seen in classical psoriasis. A deterioration in the skin condition is usually accompanied by fever, leukocytosis and a high ESR. She has never had symptoms or X-ray changes of psoriatic arthropathy, and has no nail deformity.

In October 1972, the patient was admitted for treatment of a severe exacerbation of her skin condition and was found on routine investigation to have a urinary infection and proteinuria of 2·6 g in 24 h. Because of persisting proteinuria she was referred for full renal investigation. At this time she had a blood pressure of 110/70 mmHg and no peripheral oedema.

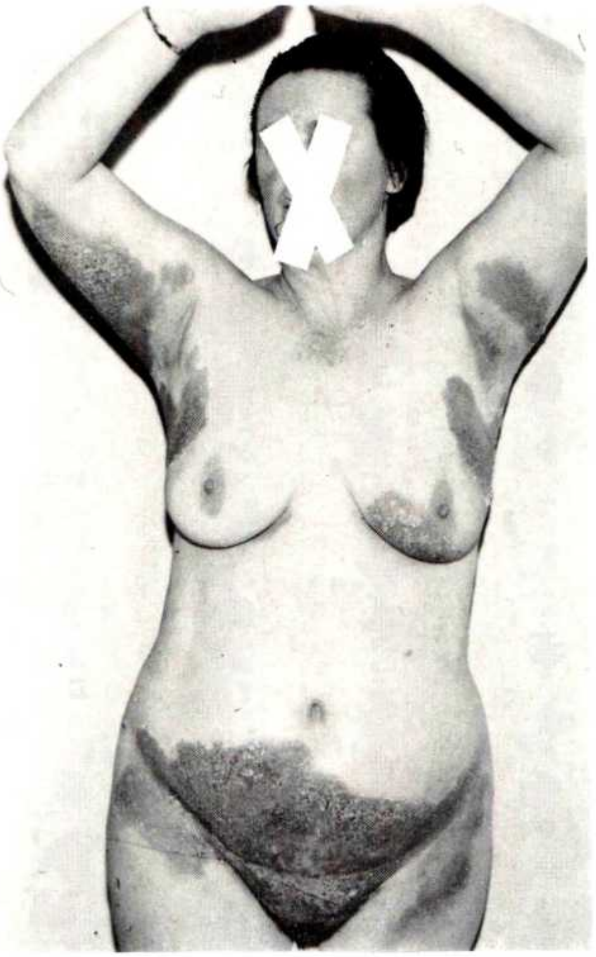

FIGURE 1. M.K. Showing areas of continual involvement. Photograph taken when disease clinically quiescent.

Abnormal haematological findings at this time were an ESR of 126 mm in the first hour and a polymorphonuclear leukocytosis with a white count of $12{,}900/mm^3$. Serum urea was 12 mg/100 ml, serum creatinine 0·8 mg/100 ml and creatinine clearance 132 ml/min. Proteinuria persisted at 2·7 g/24 h. An MSU showed no growth of organisms, and cultures of urine for acid fast bacilli were negative. An Addis count showed excess white and red blood cells but no casts. Testing of urine for Bence-Jones protein was negative.

Serum albumin was low at 2·1 g/l with a globulin level of 3·9 g/l. Serum protein electrophoresis revealed a raised α_2 globulin. Serum immunoglobulin levels were within the normal range.

Investigations yielding normal results included serum calcium, liver function tests, serum uric acid, antinuclear factor and LE latex slide test.

Intravenous pyelography revealed no abnormality, and the kidney lengths were 15 and 16 cm. Renal biopsy showed amyloid to be present in the perivascular and peritubular interstitial tissues. No glomeruli were present in the biopsy specimen. A skin biopsy stained with Congo red and crystal violet this time showed no evidence of amyloid deposition in the dermis.

A wide range of topical and systemic therapeutic agents has been prescribed for the patient's skin condition. Low concentrations of tar in zinc paste have been the mainstay of topical treatment, and in addition, antibiotic and corticosteroid preparations have been applied for short periods with temporary benefit. Systemic therapy has included a 4-month course of prednisolone in 1966 at a time when the condition was extremely active. While she was receiving this treatment, there was some improvement in the patient's skin condition, and there was no subsequent deterioration when steroids were withdrawn. Systemic antibiotic therapy has been given on several occasions for urinary tract infections, and on two occasions when *Staphylococcus albus* was isolated on blood culture. Improvement of skin lesions has frequently been observed while the patient has been receiving antibiotics. She is currently receiving ampicillin 250 mg four times a day and topical applications of 1% ichthyol in zinc paste to flexural areas.

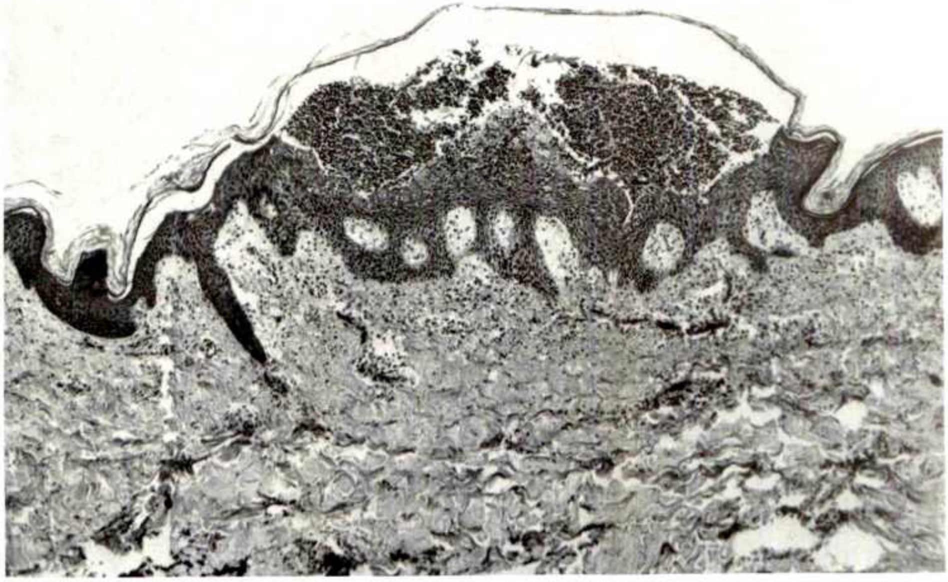

FIGURE 2. Section taken from a lesion in active stage of disease. A subcorneal pustule is clearly shown with underlying acanthosis.

DISCUSSION

The diagnosis of annular pustular psoriasis in this case is based both on clinical and on histological features. Clinically, the lesions have frequently presented as annular areas of superficial pustulation associated with fever, leukocytosis and an elevated ESR. Features of interest include the complete absence of classical psoriatic lesions, or of nail dystrophy. Arthropathy, a feature found in a third of patients with long-standing pustular psoriasis, has not been found in this case (Baker & Ryan, 1968). The exacerbation of the condition in the patient's second pregnancy raises the problem of the relationship of pustular psoriasis to impetigo herpetiformis, but in this case the length of time for which the lesions have persisted, and the repeatedly normal serum calcium levels, make the latter diagnosis untenable. The complete absence of lesions on the hands and feet eliminate the possibility of acrodermatitis continua. Repeated biopsies of areas of involved skin have confirmed the subcorneal localization of the pustule and have shown in addition some rete ridge thickening with scattered areas of acanthosis and parakeratosis, as is seen in classical psoriasis. Little histological information is available in the literature on pustular psoriasis, but it is generally considered that, apart from the striking subcorneal pustulation, some of the features of classical psoriasis may be present. No other reference in the literature to the appearance of the capillaries in pustular psoriasis, in the absence of classical lesions, has been found, and it may be that the dilatation and tortuosity of the capillaries aid escape fo the leukocytes and pustule formation.

The isolation of *Staphylococcus albus* from blood culture on two occasions, with a sterile swab from the venepuncture site, is of note in view of the findings of McFadyen & Lyell (1971). However, numerous additional cultures have been carried out during episodes of fever and leukocytosis with negative results. Nevertheless, the repeatedly observed improvement in skin lesions while the patient was receiving antibiotic therapy may indicate that bacteriaemia and leukocytosis are aggravating factors in the development of skin lesions.

The possible relationship of severe or long-standing psoriasis to the development of systemic amyloidosis has been recognized only in recent years. Cohen (1967), in an extensive review of amyloidosis, makes no reference to psoriasis as an aetiological factor. Moise *et al.* (1965) reported a case of fatal renal amyloidosis in a young man with a 3-year history of psoriasis and arthritis, and further cases have been reported since then by Berger (1969), Brownstein & Helwig (1970), and Ferguson & Downie (1969).

In four of these cases, the cause of death was rapidly progressive renal failure, and the diagnosis of renal amyloid was not firmly established until autopsy. In contrast, Ferguson & Downie (1969) described a 63-year-old woman with gross psoriatic arthropathy and unequivocal evidence of extensive gastro-intestinal amyloid deposition. There was no evidence of renal involvement in that the blood urea was normal and there was no proteinuria.

Our own patient had a 27-year history of annular pustular psoriasis. Renal biopsy has shown definite perivascular and peritubular deposits of amyloid. Unfortunately, no glomeruli were present in the sections submitted for special staining, but the presence of proteinuria of 2·4 g/day shows that the glomeruli also are affected. No evidence was found of any other underlying disease process, such as tuberculosis or multiple myeloma, to account for the presence of amyloid.

It is of interest that, including our own patient, four of the six reported cases have been diagnosed in one hospital. Although amyloidosis must be a fairly rare complication of psoriasis, it is possible that, particularly in those with severe forms of disease, the incidence of amyloidosis is higher than is at present recognized.

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Painful piezogenic pedal papules

R.R.M.HARMAN AND CYNTHIA N.A.MATTHEWS

Department of Dermatology, United Bristol Hospitals, Royal Infirmary Branch, Bristol BS2 8HW

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SUMMARY

A case of painful piezogenic pedal papules is reported. The histological findings are compatible with the hypothesis that the papules are caused by herniation of fat into the dermis.

Painful piezogenic pedal papules were first described by Shelley & Rawnsley (1968), who suggested they were caused by herniation of fat into the dermis. Neither these authors nor Cohen *et al.* (1970) nor Galinski (1970) described the histology of the lesion.

Woerdeman & Van Dijk (1972) recently reported six cases, and described the histology of three papules. Their biopsy specimens showed hyperkeratosis, a normal epidermis with slight acanthosis, and apparently normal dermis and subcutaneous tissue. In one case, the small blood vessels in the mid-dermis showed slight hyaline thickening of the walls. In that biopsy the subcutaneous fat appeared located unusually near to the epidermis, at one point around a sweat duct. The collagen, elastic and reticular fibres were normal. There was some oedema of the dermis. The biopsy specimens in this case were taken with the patient standing.

In none of these papers was an illustration of the histology published, and at least one very experienced skin histopathologist has never had an opportunity to examine a specimen (Wilson Jones, 1973).

Recently a patient presented in Bristol with painful papules on the heels as described by Shelley & Rawnsley. The patient, a 56-year-old white female, gave a 2-year history of several papules on both heels, which were exquisitely tender, even when lying in bed. She also had valvular heart disease with atrial fibrillation following rheumatic fever in early life. She agreed to biopsy of one of the papules during a general anaesthetic for dental clearance.

The papule had previously been marked on the skin when she was standing, but was, in fact, still visible when she was anaesthetized in a supine position. A wedge biopsy, including the papule and underlying subcutaneous tissue, was obtained. Macroscopically, the specimen showed a well defined nodule, 0·4 cm in diameter, in the dermis; the cut surface was yellowish and translucent. The overlying epidermis was normal. Histological examination (Dr O.C.Lloyd) showed the nodule to be composed of mature adipose tissue, the deeper layers of which were compressed. The dermis overlying the nodule was reduced in thickness to half that on either side of the lesion. There was a thin fibrous capsule surrounding the fat nodule.

Although Shelley & Rawnsley postulated herniation of fat into the dermis as the cause of painful

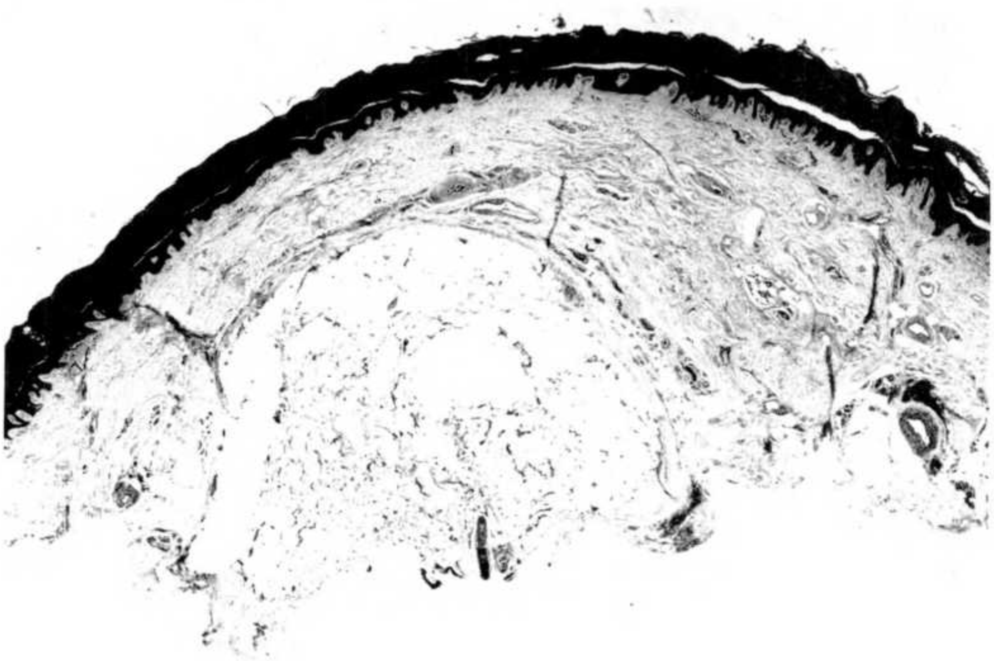

FIGURE 1. Histology of pedal papule clearly showing the protrusion of an encapsulated fatty nodule into the dermis (H & E, × 17·6).

piezogenic papules of the heel, this appears to be the first time that this theory has been supported histologically. There was no evidence of fat necrosis, vascular change, or of inflammation, and the cause of the associated pain is obscure.

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Pemphigus and Hodgkin's disease

V.D.SOOD AND J.S.PASRICHA

Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi-16, India

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SUMMARY

The coexistence of pemphigus and Hodgkin's disease has not previously been reported. We observed a patient who developed pemphigus vulgaris nearly 2 years after Hodgkin's disease had been found to be present. Subsequently the diseases ran a parallel course in three relapses and remissions. The remissions were induced by cyclophosphamide. The possibility that Hodgkin's disease predisposed the patient to pemphigus is considered in view of the recent postulate that thymus-dependent lymphocytes prevent the development of autoimmunity, and that the diseases associated with depression of cell mediated immunity can lead to the development of autoimmune phenomena.

The presence of autoantibodies in the sera of pemphigus patients has generally been accepted to mean that pemphigus is an autoimmune disease. But so far nothing is known about the circumstances which lead to the production of these antibodies. Recently, Allison, Denman & Barnes (1971) have postulated that thymus dependent lymphocytes play a key role in preventing autoimmunity, and that diseases associated with a depression of cell mediated immunity can lead to the development of an autoimmune process. The depression of cell mediated immunity in Hodgkin's disease is well recognized, and several autoimmune diseases have been reported to occur in patients suffering from this disease (Brown & Meynell, 1949; Willcox, 1952; Kyle, Kiely & Stickney, 1959; Eisner, Ley & Mayer, 1967; Hench *et al.*, 1962; Cammarata, Rodnan & Jensen, 1963).

To the best of our knowledge, pemphigus has not so far been reported in a patient with Hodgkin's disease. We had the opportunity of observing a patient with Hodgkin's disease, who developed pemphigus, and in whom thereafter the diseases seemed to run a parallel course.

CASE REPORT

The patient was a 24-year-old farmer who, in February 1969, noticed asymptomatic 2–3 cm swellings in the submandibular region. Within a month, similar swellings appeared in both axillae, groins and neck, and gradually increased in size to 6–7 cm in diameter. At this stage, he started having a low grade irregular fever. Histology of a cervical lymph node, biopsied in another hospital, suggested Hodgkin's disease and the patient was given treatment, after which the swellings subsided. During the next 2 years, he had three such episodes of lymphadenopathy which were treated in the same manner.

In February 1971, he started developing bullous lesions on the face, and later on the scalp, and on the trunk and extremities. In April 1971, when he first came under our care, he had multiple, diffusely scattered bullous lesions all over the body. Small lesions were tense, while larger ones were flaccid. There was no erythema around the lesions. The mucous membranes were not involved. Nikolsky's sign was positive, and a Tzanck smear showed the presence of acantholytic cells. In addition, he had enlargement of multiple groups of lymph nodes in the cervical, axillary and inguinal regions on both sides, their size varying between 1 and 3 cm. They were non-tender, discrete, and rubbery in consistency. The liver was palpable 2 cm and the spleen 3 cm below the costal margin. Both were firm, smooth and non-tender.

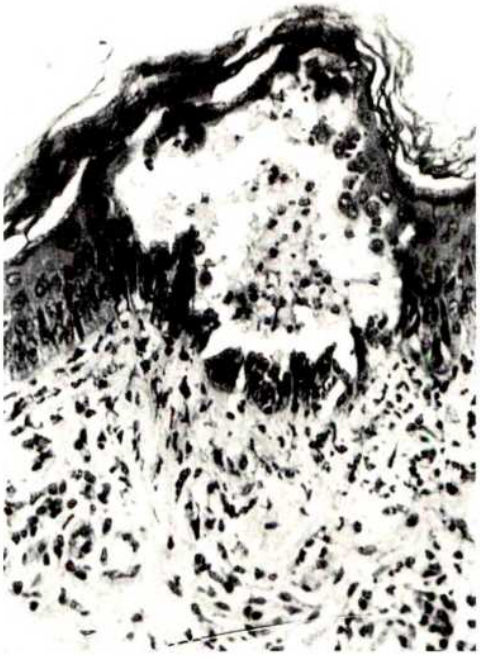

FIGURE 1. Intraepidermal vesiculation with typical acantholytic cells (H & E, × 200).

Biopsy of a fresh bulla (Fig. 1) showed an intraepidermal split with acantholytic cells. The serum showed the presence of antibodies against an extract of normal human epidermis, by the passive cutaneous anaphylaxis test (Pasricha, Mathur & Kandhari, 1972). The histopathology of a cervical lymph node (Fig. 2) showed obliteration of the follicular pattern of the node by a pleomorphic infiltrate, consisting of atypical reticulum cells interspersed with lymphocytes, polymorphs and a moderate number of eosinophils. In some fields, Sternberg–Reed cells were also seen. Peripheral blood counts were normal, and radiological examination of the chest and abdomen showed no evidence of lymph node enlargement. On the basis of the enlargement of multiple groups of lymph nodes, liver and spleen, with the presence of low grade intermittent fever, and with the histological findings, the diagnosis was made of Hodgkin's disease associated with pemphigus vulgaris.

The patient was treated with prednisone 60 mg daily, increased to 80 mg daily after 15 days; but new bullae continued to appear intermittently for 2 months. In July 1971, intravenous injections of cyclophosphamide on alternate days were started. The lymph nodes began to regress and the skin

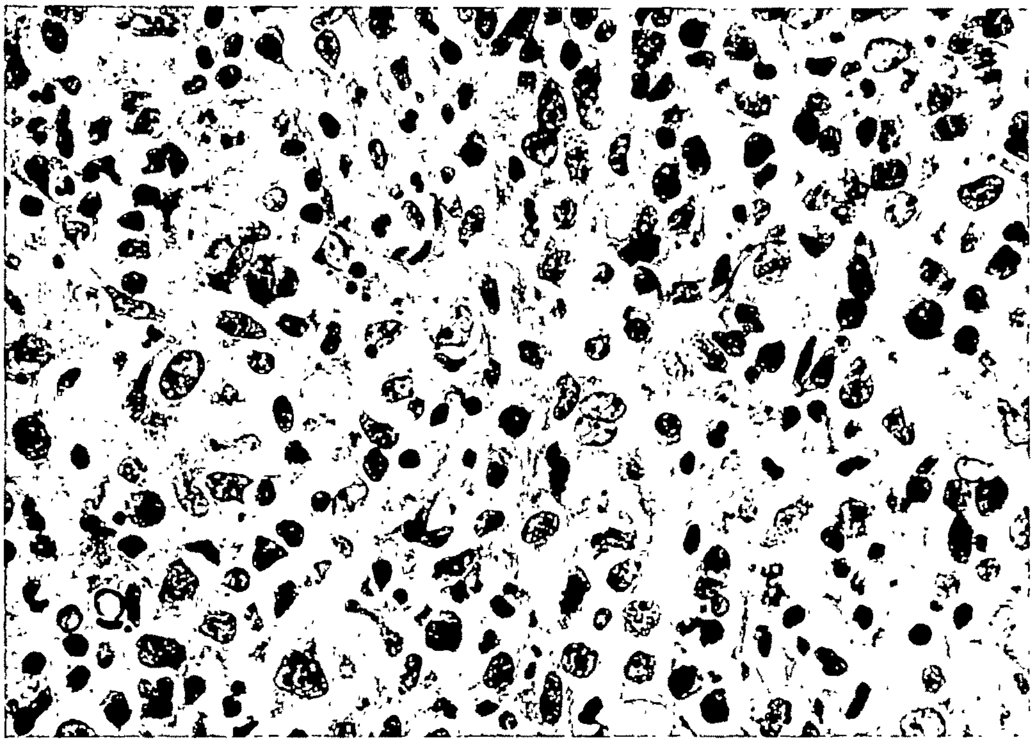

FIGURE 2. A section of lymph node showing lympho-reticular tissue containing large atypical reticulum cells, some of which have prominent nucleoli. The overall appearance was characteristic of Hodgkin's disease. (× 400).

lesions healed in 22 days (total cyclophosphamide 2·2 g). During this period the dose of prednisone had been gradually reduced to 10 mg daily and this was continued with oral cyclophosphamide 50 mg daily for a further month.

In November 1971, the lymph nodes started enlarging again and the bullae recurred. This time he was treated only with cyclophosphamide 200 mg intravenously daily for 20 days (total 4·0 g). The lymph nodes regressed within a week while the skin lesions responded in 2 weeks. He was then started on prednisone 20 mg daily, the dose being progressively reduced during the next month.

In January 1972, there was another recurrence both of lymph node enlargement and of skin lesions, and he was again treated with cyclophosphamide 200 mg intravenously daily for 20 days, with regression of lymph nodes and complete healing of skin lesions.

DISCUSSION

The immunological system of a healthy individual shows tolerance to 'self' antigens. Kaplan (1962) has, however, hypothesized that this tolerance can be broken if the individual is immunized with a foreign antigen which has antigenic determinants in common with the 'self' antigen. Beutner, Jordan & Chorzelski (1968) put forward this hypothesis to explain the occurrence of intercellular antibody in very high titres in patients with Fogo Selvagem (pemphigus foliaceus of Brazil), which is believed to be caused by an arthropod-borne infective agent.

Miller (1967) has proposed that a lymphoma represents an unrestricted and uncontrolled proliferation of the cells which constitute the immunological system. Therefore, the emergence of a 'forbidden' clone which can produce autoantibodies is not unexpected. This would account for the occurrence of autoimmune diseases in patients suffering from lymphomas. An example of such an occurrence in pemphigus has been reported only recently (Saikia, 1972), where pemphigus developed in a patient with a giant follicular lymphoma. At autopsy, the tumour was found to be producing large quantities of pemphigus autoantibody.

Another explanation for the appearance of autoantibodies in an individual is the emergence of 'forbidden' clones during spontaneous mutations. Such clones are, as a rule, eliminated by the homeostatic mechanisms in the body. But where an homeostatic mechanism fails, the persistence of 'forbidden' clones and occurrence of autoimmune diseases can be expected. The hypothesis of Allison *et al.* (1971) that it is the thymus-dependent lymphocytes (T-cells) which prevent the emergence of autoimmune disease, implies that autoantibodies can develop more easily in association with those diseases in which cell mediated immunity is impaired. This hypothesis has been substantiated by the frequent occurrence of autoimmune phenomena in patients suffering from Hodgkin's disease (Brown & Meynell, 1949; Willcox, 1952; Kyle *et al.*, 1959; Eisner *et al.*, 1967; Hench *et al.*, 1962; Cammarata *et al.*, 1963) or lepromatous leprosy (Matthews & Trautman, 1965; Wager, 1969), but so far pemphigus has not been reported in association with either of these diseases. Our case appears to be the first reported instance of such an association. In the majority of patients with pemphigus, however, there is no evidence of any of these predisposing factors. Search, therefore, has to be continued for other mechanisms which favour the production of autoantibodies.

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Comment

A NEW ROUTINE IN HISTOPATHOLOGY

Histopathologists are largely restricted to establishing diagnoses by means of studies on artifacts under the light microscope. Two kinds of artifacts may exist:

(1) Positive artifacts which are supposed to reflect conditions *in vivo*.

(2) Negative artifacts which are produced by chemical, thermal or mechanical insults to the tissues.

The positive artifacts are informative in diagnostic work, but the negative ones are usually detrimental in that they interfere with the interpretation of tissue changes. Positive artifacts may be exemplified by the nuclear staining reactions in histology, and by the coloured precipitates, indicating enzyme activities, in histochemistry. Negative artifacts may be represented by the loss of stainable substances and by morphological defects usually from chemical and thermal damage during paraffin embedding, and diffusion problems and mechanical defects during freezing and thawing of tissues in enzyme histochemical studies.

The methods used today in routine histopathology are extensively based on fixation and paraffin embedding of the tissues. Most of the procedures have been unchanged for many decades. The reluctance to introduce variations in the laboratory routine maintains the continuity of criteria for morphologic evaluation of tissue changes and thus simplifies the training of pathologists.

NEED FOR STANDARDIZATION

It is evident that the more data can be provided from a given lesion than the more reliable may be the diagnosis. The number of light microscopic criteria in the different diseases is limited, when they are recorded in terms of conventional histology alone. It follows that if the individual biopsy could supply further relevant information the accuracy of differential diagnostic work should be increased.

The supplementary techniques available today include light microscopic histochemical, and electron microscopic morphological and histochemical analyses. Unfortunately, the conventional tissue fixatives and subsequent paraffin embedding make such analyses difficult or even impossible. Biopsies are daily arriving at histopathological laboratories in different types of fixative, in which they have been immersed for varying periods and at a variety of temperatures. These factors are usually difficult to control when a service is provided in institutions outside hospitals or faculties. Such conditions of transport promote various positive and negative artifacts in the tissues. Since all differential diagnostic work using the light microscope is based on comparative evaluation according to traditional morphological criteria, variations in the treatment of tissues outside the laboratory should be avoided. Otherwise no standardized staining results and artifacts can be obtained. Ideally, the pathologist should carry out or control the fixation of the biopsy.

TISSUE TRANSPORT

In order to obtain most quickly the largest possible amount of information from a biopsy with light microscopic techniques available today, combined histological, histochemical and enzyme histochemical studies would be practicable. Standardized transport and laboratory procedures may then combine to increase the number of diagnostic criteria in each disease. Data on the use of histochemistry in routine differential diagnostic work are accumulating in the literature. Most of such work is based on fresh frozen tissues. However, fresh frozen specimens are difficult to obtain routinely. Technical freezing arrangements within the operating theatre and transport at very low temperature are two of the problems involved in such procedures. The difficulties are even more obvious when remote institutions are served. The easiest method would be to use a tissue preserving transport medium, without a strong fixative, for transport at room temperature when histology alone is desired, and at a lower temperature (around +4C°) when enzyme activities also must be studied. Well-controlled time and temperature factors, employing one common medium for all types of studies facilitate standardization of artifact production. The clinicians then decide, in agreement with the pathologists, at which temperature transport of the individual specimen should be carried out, but the pathologist alone decides on the type and duration of fixation, and on which sectioning technique should be used. The range of choice laboratory technique is thus increased.

Since 1971, all diagnostic biopsies have been sent to our laboratory in a special transport medium (Heyden *et al.*, 1972), containing both a buffer (cacodylate) and a colloid stabilizer (polyvinylpyrrolidone) which are employed in routine tissue preparation for electron microscopy. An antimicrobial and protein preserving component (chlorhexidine) inhibits autolysis and bacterial growth during transport. The medium has been named Histocon (Histo-Lab, Göteborg, Sweden). It is hypertonic (591 mosm) to serum but much less hypertonic than ordinary fixatives. Histocon has an almost neutral pH (7·2). Biopsies transported in this medium can be fixed, paraffin embedded or frozen without any preliminary washing. The pathologist may determine which techniques are to be employed after the arrival at the laboratory of the unfixed specimen.

Tissues transported at room temperature can be utilized for conventional histological and histochemical studies after paraffin or cold microtome sectioning. For such purposes the duration of immersion in Histocon has been found to be unimportant.

Tissues transported at low temperature (0–4°C) are especially suited for cold microtome sectioning and hence for histochemistry, for enzyme histochemistry and, under certain circumstances (Arwill, Lilja & Svensson, 1973), also for electron microscopy. All these procedures can, thus, be performed on the same biopsy. When fixation is regarded as necessary prior to staining, this is carried out only on tissue sections, while the rest of the specimen is still intact in the freezer for future studies, e.g. for supplementary histochemical techniques. This routine thus greatly increases the possible use of biopsy material in diagnosis, research and education.

ROUTINE

At our laboratory we use only cold microtome sectioning of tissues transported in Histocon at low temperature or at room temperature. The tissues are mounted on modified cryostat chucks and frozen in isopentane or propane–propene, which are chilled close to their freezing point by liquid nitrogen. Prior to sectioning, the frozen tissues are stored at −70°C. Sections are cut in cryostats (System Dittes Duspiva, Heidelberg, Germany) at −20°C. The sections are placed on glass slides covered with a gelatin film. To obtain the morphological appearances necessary for diagnosis, some of the artifacts known from conventional fixation and paraffin embedding techniques can be induced in the cold microtome sections. This can be achieved by immersion, prior to histological staining, of the sections for 5 min in, for example, 4% neutral buffered formalin and thereafter in graded alcohols up to absolute alcohol and back to distilled water. Sections intended for histochemistry, for example for staining of lipids or acid mucopolysaccharides, can be fixed for 5 min in formalin vapour in a refrigerator. This treatment increases the attachment of the frozen sections to the surface of the glass slides. Neither histology nor routine histochemistry seem to be critically dependent on time and temperature factors during biopsy transport in Histocon (Eldh, Bartholdsson & Mobacken, 1974).

For enzyme histochemical studies, the cold microtome sections (8 μm) can be stored for up to 24 h in the cryostat (at −20°C). When removed from this low temperature the sections must immediately be incubated in media freshly mixed or must be thawed. Hydrolytic enzymes seem to survive Histocon treatment in the cold for at least 3 weeks. The oxidative enzymes, however, are more susceptible and, in order to retain an activity which is visibly comparable to that in fresh frozen controls, the cold transport should not exceed 48 h (Heyden *et al.*, 1972).

Electron microscopic morphological and enzyme histochemical tests (e.g. for acid phosphatase) can be performed on the frozen tissues, provided the cold transport in Histocon has not lasted for more than 10–15 h (Arwill *et al.*, 1973). Sections of 40–60 μm are cut in the cryostat and thereafter fixed, embedded in Epon and stained routinely.

DISCUSSION

Diagnoses based on morphology alone may be established within a few hours after the arrival of the biopsy at the laboratory, owing to the rapidity of the cold microtome methods. Specimens larger than 1 × 1 cm may be cut into smaller pieces and mounted on individual chucks.

The increased expense of the tissue transport as compared with that of conventional methods may be justified by the much increased possibilities for investigation of the tissues. Thus more criteria may be available on each disease, for differential diagnostic work. Additional biopsies for supplementary microscopic studies beyond routine histology are unnecessary. The methods described allow routine diagnostic work to be integrated with research work, an advantage which compensates for some of the additional expense involved.

Cryostat chucks are usually rather expensive and space consuming but both of these drawbacks can be diminished by mounting the tissues on thin blocks of brass, which fit to an adapter in the cryostat, thus allowing storage of thousands of biopsies in each freezer (at −70°C). Diagnosed specimens which seem to be of little interest for further study may be thawed, fixed and embedded in paraffin for future storage at room temperature.

The laboratory work can largely be rationalized after necessary initial tests, including adaptation of staining solutions and staining times.

Most enzyme histochemical incubation media may be frozen in suitable batches and stored until enough tissue specimens have accumulated to be submitted to enzyme activity evaluation. The enzyme histochemical studies are usually performed once a week on selected biopsy specimens.

In our laboratory, routine histological staining with haematoxylin and eosin and with the van Gieson technique is regularly supplemented with histochemical staining for keratin, lipids, sulphated acid mucopolysaccharides and PAS-positive compounds. All oral manifestations of skin diseases and all potentially malignant or established malignant oral, skin or vulval lesions are submitted also to enzyme histochemical tests. In this way the presence of, for example, metabolic atypia (Heyden, 1973), if demonstrated, may confirm a morphological suspicion of malignancy. The methods have been found to be especially suited for classification of pigmented skin lesions such as the different types of malignant melanoma (unpublished data), and for the evaluation of therapeutic effects on tumour tissue (Overgaard, Heyden & Arwill, 1974).

Department of Oral Histopathology,
Faculty of Odontology,
University of Göteborg,
Fack,
S-400 33 Göteborg 33,
Sweden

GUY HEYDEN
TORE ARWILL
MARIA HÖGBERG

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Obituary

D.C.MACMILLAN

Duncan Campbell Macmillan, aged 36, died with tragic suddenness on 13 December 1973, 3 months after being appointed Consultant Dermatologist to the Derbyshire Royal Infirmary. He was the third son of the late Dr Duncan Macmillan, Consultant in Charge of the Mapperley Hospital, Nottingham, who was one of the forerunners of the modern approach in psychiatric therapy.

Macmillan was educated at Sedbergh and Edinburgh University where he was an active forward in the 2nd rugby XV. He qualified in 1960, having a distinguished academic career, and held several house appointments int he Edinburgh Royal Infirmary before becoming Assistant Lecturer and British Heart Foundation Research Fellow in the Department of Medicine of Edinburgh University. He was particularly interested in problems of blood flow, and he worked on platelet aggregation. At this time he obtained M.R.C.P. (Ed.). However, he realized that his main love was clinical medicine, and his interest in dermatology had been established both as a student and while a resident in Edinburgh. He was appointed Registrar in the Department of Dermatology in Oxford where he was able to continue his work on platelets and successfully submitted a thesis on this subject for the M.D. He was always a hard worker, and possibly his one failing was that of setting himself too high a standard of excellence.

When a vacancy occurred for a Senior Registrar in Oxford, he was appointed to the post, since it was evident that he would make a good Dermatologist, and had the inquiring mind and the general medical and research background necessary to advance knowledge in diseases of the skin.

He presented many patients to various Societies and went to the trouble of having elocution lessons to improve the carrying power of his rather soft voice. He was well liked by patients, nursing staff and colleagues. When a Senior Registrar post was established in the new Teaching Centre at Nottingham, he took this opportunity of returning to the North Midlands, and was appointed there 2 years ago. There he worked hard, but went through a phase of indecision about where he wished to settle as a Consultant. However, eventually he was very happy to be appointed to Derby. The day before he died, he had been discussing the future running of the department there with his colleagues.

The Minister said at the very moving funeral service that 'we do not know the answer to their problems, but we must be thankful for the skill and care he gave to his patients'.

Our sympathy goes to his wife, an Edinburgh nurse, and his two children.

RENWICK VICKER

Correspondence

KERATINIZATION OF SUBCULTURED KERATINOCYTES

SIR, We have read with great interest the paper by Bauer & De Grood (1973). The authors described the successful induction of horny layer formation from 'fibroblast-like' cells (Fig. 1). In the photographs a thick stratum corneum is shown (Fig. 3), which is said to be produced in 4 days. We detected at least twenty horny cell layers in this figure. The rate of corneocyte formation in these cultures therefore seems to be exceedingly high, namely five cell layers per day. Even if one considers *in vivo* conditions, under which eight to ten basal cells are covered by one corneocyte, it would take 48 h to replace these ten basal cells. This gives a rate of new cell production of one cell per 4·8 h. Such a value has been observed in monocellular organisms, but not in mammalian cells. One must therefore question the production of this enormous number of well stratified horny cell layers in such a short time. Our own experiments have failed to achieve horny layer formation or such a high proliferative rate.

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G.PLEWIG
E.CHRISTOPHERS

Department of Dermatology,
University of Munich,
8 Munich, 2, Frauenlobstr. 9,
Germany

SIR, We would like to give our comment on the letter from Plewig and Christophers. We think that basically there is only a simple misinterpretation of our results.

As we mentioned in the paper, aggregates of subcultured keratinocytes keratinized within 4 days. It is quite certain that the cultural conditions (on top of a millipore filter) did not promote multiplication of the cells. Most probably therefore the *in vivo* situation in which a basal cell layer has a certain transition time before it keratinizes, and in which the thickness of the epidermis is kept constant by some feedback mechanism, did not exist.

We think that the 'multilayered' arrangement perhaps starts to keratinize on its outer surface and then gradually downwards towards the filter, as some basophilic non-keratinized cells are still present. Thus, when twenty horny cell layers are visible in the photograph, this means only that the aggregate consisted of at least twenty cell layers. Consequently there is no need to postulate a cell cycle of 4·8 h; the cells have taken approximately 4 days to keratinize.

The aim of the experiment was not to present a method for reconstituting epithelium, but to show that it is in principle possible for a subcultured keratinocyte to differentiate into a corneocyte.

Department of Dermatology,
University of Nijmegen,
The Netherlands

F.W.BAUER
R.F.DE GROOD

ATYPICAL SCALP RINGWORM CAUSED BY *MICROSPORUM CANIS*

SIR, *Microsporum canis* infections are the main sort of ringworm of the scalp seen in white children living in the Transvaal. Mostly they follow the clinical phases set out by Kligman (1955), but some 3% of cases break the rules, and have caused errors in diagnosis and delay in treatment.

The one type, of which I have now seen eight examples, has particularly affected girls between 3 and 8 years of age, and consists of extensive pityriasiform and psoriasiform patches on the scalp with minimal hairfall. The unbroken hairs fluoresce extensively. The complaint had lasted from 8 months to over 2 years and may have started with a short phase of ring-shaped hair loss followed by regrowth with persistence of the hair and scalp infection. The patients themselves and the organism isolated were otherwise unremarkable, and the response to treatment with griseofulvin was quite as satisfactory and prompt as usual. The source of infection was usually a cat. When other children in the family were infected from the same animal, the scalp ringworm followed the usual clinical pattern.

Such occurrences make it essential to examine under Wood's light chronic scaling conditions of the scalp without hairfall in children, particularly when there are no lesions diagnostic of psoriasis or seborrhoeic eczema to be discovered elsewhere. Neither the long duration nor the absence of hairfall could be used as arguments against the possibility of *M. canis* scalp ringworm, contrary to prevailing teaching.

The other type is equally long lasting but less common, and consists of episodes of superficial scalp suppuration with matting of the ringworm hairs, resembling a surface impetigo rather than kerion. The lesions become painful and undergo surface necrosis; the crusted bunch of hairs comes out together with part of the scalp, leaving an angulated cicatricial alopecia. The latter is surrounded by hairs still infected with *M. canis*. I have met with three such cases.

My experience therefore indicates that the strict divisions between favus-like changes, pseudopelade, teigne fausse, teigne tondante, suppurative tineas, etc., are obliterated in these rare examples of *M. canis* infection. There are hints that these features may have been noted before, but have slipped out of currency (Rabut, 1953). Such changes would be perfectly in order with various species of trichophyton infections. Some of the *M. canis* cases possibly owed their peculiarities to inadequate dose and duration of griseofulvin treatment in the early stages, but others had had no griseofulvin.

I wish to thank Dr de B.Scott and Mrs H.F.Vismer for the mycological studies.

G.H.Findlay

Section of Dermatology,
University of Pretoria,
Pretoria,
South Africa

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NAIL ANOMALIES IN HODGKIN'S DISEASE

Sir, I was interested in the nail changes described by Shahani & Blackburn (1973) in their four patients with Hodgkin's disease. The most striking feature of the nails illustrated in the colour plate, is the brown pigmented arc affecting the distal part of the finger nail bed. Dr W.K.Stewart and I (1972) drew attention to a 35% incidence of such pigmentation in chronic renal disease compared with an incidence of less than 2% in a general hospital population; we discovered only seven such patients over the course of a year among 500 or more whose nails were carefully inspected. Three of these seven showed evidence of renal impairment while four did not, although one of these four had a urinary infection and hypothyroidism. Two others had biliary cirrhosis without the white nails often seen in cirrhotic subjects (Terry, 1955), and the fourth patient was an obese mildly diabetic subject; such pigmentation is therefore not specific for renal disease. I cannot recall that we examined the nails of any patients with Hodgkin's disease in our hospital population, but it would be interesting to know about the renal function of the patient whose nails are illustrated in Shahani & Blackburn's article.

We have postulated that the brown arc in the nail bed may be in a site where certain types of acquired pigmentation are manifested early. Whether the brown pigment of the arc is lipochrome, melanin, or some other unknown pigment is not clear. The location of the brown arc near to but not beyond the free margin of the nail suggests that the arcs may be the result of a process similar to the oxidative darkening of skin lipochromes known to occur in some cases of uraemia (Tsaltas, 1969), but there was no association with general skin pigmentation in our series of patients with chronic renal disease.

E.J.Raffle

Department of Dermatology,
The University,
Dundee

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THE PERSISTENT NUCHAL OR OCCIPITAL SALMON PATCH

SIR, Newborn babies often show one or more telangiectatic naevi (salmon patches or 'stork bites') due to localized capillary dilatation. They present at birth or soon after as pink stains, which are often inconspicuous, over (1) the occipital region or nape, (2) the mid-forehead above the nose, (3) the eyelids. Those on the eyelids and forehead usually subside very rapidly, and certainly within a year, but there is much difference of opinion regarding the fate and indeed the incidence of the nuchal or occipital naevi.

As regards the incidence of the latter naevi in the newborn, Domonkos (1971) gave a figure of 5%; Smith & Manfield (1962) found an incidence of 27% and mentioned that Pollitzer's (1898) figure was 35%. Bettley (1940) found an incidence of 62%, whilst Sulzberger, Wolf and Witten (1961) said that they are so common that they must be considered almost a normal or physiological variant.

Lumping together the fate of salmon patches in all three areas, Illingworth (1973) said that they always disappear in a few months, whilst Davies *et al.* (1972) said that they nearly always disappear. Sneddon & Church (1971) commented that the majority of facial lesions fade in the first few months of life, and they added that the occipital area is hidden by hair!

Smith & Manfield (1962) traced at 1 year some of their babies who showed a nuchal patch in the first week of life, and they found the patch persisting in just over half (56·5%). Øster & Nielsen (1970), in a study of Danish children aged 6–17 years, noted nuchal naevi in 41%, and Bettley (1940) found a persisting patch in 50% of a group of patients whose ages ranged between 5 months and 57 years.

We have examined 188 hospital in-patients and old-age home residents, aged 60 years and over, for the presence or absence of a salmon patch over the occiput or nape. The patients examined were from general medical wards and many were long-stay geriatric patients. Patients with known skin conditions were excluded. The 188 patients consisted of sixty-seven males and 121 females. The age range for males was 60–97 (mean 78) and for females was 60–108 (mean 80).

Forty (60%) of the males and fifty-one (42%) of the females showed typical nuchal patches. The lesions were clearly-defined, macular, pink to purplish-red patches of various sizes, present over the nape and/or in the occipital hairline. The naevus sometimes consisted of small macules rather than one confluent patch.

From our findings in the aged, and from those of Øster & Nielsen (1970) and Bettley (1940) mentioned above, it seems clear that salmon patches over the nape of the newborn are very common and usually persist. As 48% of the elderly persons examined still showed them, it is possible that a much higher percentage had them in infancy.

It is, however, possible that nuchal naevi occasionally appear in childhood, and this could account for surveys performed on adults giving slightly higher figures for persisting patches than would be expected if the patches appeared only in the newborn. In fact, in only one of Smith & Manfield's (1962) fanints had a nuchal patch, noted during the first year of life, not been seen during the first week of life.

Again it is possible that these naevi become more prominent and easier to detect in senile skin after having becoming fainter in childhood. We are not aware of any evidence for their initial appearance in later life.

JULIAN VERBOV
RONALD STEINBERG

Newsham General Hospital,
Belmont Road,
Liverpool L6 4AF

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BETAMETHASONE AND BETAMETHASONE VALERATE

SIR, May I draw attention to what appears to be an error in nomenclature in Dr Ian Sneddon's paper, 'A Trial of Hydrocortisone Butyrate in the Treatment of Rosacea and Perioral Dermatitis' (*British Journal of Dermatology* (1973) **89**, 505).

In the table under 'Previous treatment' many of the patients are described as having used Betamethazone (*sic*). I imagine that the author means betamethasone valerate. Betamethasone (alcohol) and betamethasone valerate (an ester) are two different compounds with very different biological properties. Betamethasone valerate, as is fairly widely known, has high topical anti-inflammatory activity but relatively low systemic anti-inflammatory activity. On the other hand betamethasone has poor topical activity but is considerably more potent than betamethasone valerate systemically (Friedman *et al.*, 1967).

Now that the wide separation of topical from systemic effect in betamethasone valerate has been shown to be of considerable value in areas of medicine other than dermatology, notably asthma (Frears, Wilson & Friedman, 1973), it seems even more important to be precise in the use of names.

LYN WILSON

Clinical Research Unit,
Glaxo Laboratories Ltd,
Greenford, Middlesex

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Book Reviews

Psoriasis: Proceedings of the International Symposium, Stanford University 1971. EUGENE M.FARBER and ALVIN J.COX (Eds.) (1972) Stanford, University Press: London, Oxford University Press. Pp. 473, 122 tables, 149 figures. Price £8.75.

You have to be a gargantuan reader to keep up with the current literature these days even in a personal sphere of interest. However, I find that only a very small proportion of what is published actually imparts new information or gives genuine pleasure. Of course a book is often of value without fulfilling either of these functions—in particular if it serves as an authoritative review or as a speculative commentary on some subject.

The proceedings of the symposium held at Stanford in July 1971 on psoriasis has elements of both review and speculation between its covers. There are fifty-one articles spread over 473 pages in which approximately eighty authors have documented our ineptitude at understanding and dealing with psoriasis. The real usefulness of the sort of symposium which this book records is the informal contact and unrecorded discussion before, between and after the formal presentations. The presentation is quite attractive although the quality of the illustrations is less than perfect. Unfortunately the content is uneven. The contributions vary greatly in style, format and detail. Some merely record a piece of work already published elsewhere, while others are genuinely useful commentaries on some aspect of the disease, or supply new data. A contribution from Cats is particularly thought provoking. It deals in a critical and provocative way with the relationship between psoriasis and associated polyarthritis. This author believed that psoriasis and chronic polyarthritis occur together only by chance and suggested that there is a constitutional, perhaps hereditary, tendency in certain individuals and families to the development of both diseases.

I also especially enjoyed a chapter by Muller, Winkelmann & Vandersteen which deals with the histochemical demonstration and the distribution of Langerhans' cells and cutaneous nerves in psoriatic lesions. Nerves were found to be normally distributed, but there appeared to be a comparative decrease in the number of Langerhans' cells within psoriatic lesions.

There is a useful chaper by Ohkawara & Halprin on carbohydrate metabolism in psoriasis in which the large amount of work accomplished by this group and by others in this subject area is nicely summarized. There are a number of chapters on various aspects of therapy. Stoughton's on corticosteroids is an especially enjoyable discussion on the problem.

The scanning electron microscopy of scale from psoriatic plaques is described by Orfanos in an interesting and provocative chapter. Many other contributions contain valuable nuggets of information and it is impossible to mention them all, but it is worth giving a few more examples. There is a neat little piece of work by Feibleman, Dobson & Dobson on sweating in psoriatic lesions. These workers found that stripping the psoriatic plaque with adhesive tape led to increased sweating and an increased concentration of sodium in the sweat. They ascribed this to the removal of poral obstruction and contamination by serum.

Baker gave a handy summary of the pustular psoriasis situation and Cox & Watson provided a helpful piece on the histology of psoriasis, pointing up the differences in histology with different stages of the disease.

The genetics of the disease are very well covered in a number of papers that come from Farber and his group at Stanford, and from other centres. It seems that there is no clear cut genetic mechanism; psoriasis is apparently inherited 'multifactorially' and is not due to a single gene.

This book is for those particulary addicted to psoriasis. It will serve as a reference point for what was known about psoriasis in 1971, but it falls short of being a major contribution because of its uneveness.

RONALD MARKS

Leitfaden der medizinische Mykologie. HERBERT KOCH (1973) Jena: Gustav Fischer. Pp. 128, 62 illustrations. Price £3.84.

The author of this slim volume is responsible for the mycological laboratory service of the Dermatology Department at Erfurt, and intends his contribution to bridge the gap between academic biology and clinical medicine. The first part (12 pages) deals with elementary botanical data at G.C.E. 'O' level standard, while the second part, which the author considers to be a comprehensive account of dermatomycoses, lists, on 22 pages, rare as well as common clinical disorders, giving little more information than most medical dictionaries and no guidance on the significance and management of the clinical problems.

Most of the remaining 70 pages, the bulk of this 'Primer of Medical Mycology', contains detailed and useful information on how to culture and identify fungi in the laboratory. There are also brief sections on mycotoxins (which should have been included in part 1) and on physical and chemical methods of destroying fungi *in vitro*, with special reference to public baths and industry.

This little book should be useful for laboratory technicians, clinical pathologists and dermatologists who wish to have a simple source of reference to dermatomycological laboratory techniques.

There are hardly any references in the text, but at the end of the book there are three pages listing relevant monographs and journals, many in English. The standard of production is austere.

GUNTER HOLTI

The Management of Common Skin Diseases. C.P.A.DUPONT (1973) London: Henry Kimpton. Pp. 86. Price £1.25.

This short work, intended for general practitioners and medical students, is full of simple useful information about the management of the more common skin conditions. It assumes that a correct diagnosis has already been made.

General principles are discussed first, followed by the more frequently used forms of treatment and their hazards, such as the modern tendency to use topical corticosteroids indiscriminately on all dermatoses. Then follow chapters on groups of conditions.

The author still considers benzyl benzoate applied for three consecutive nights to be the treatment of choice in scabies and that radiotherapy is still being used for the treatment of benign skin conditions.

While most dermatologists have long since abandoned the use of thorium X, it is recommended for the use of 'port wine stains'.

In spite of these minor criticisms, the author has packed a great deal of good common sense advice into this small pocket manual. If it were widely read and its advice heeded, much unnecessary and ill-conceived prescribing would be avoided.

NAPIER THORNE

Systematische Dermatologie und Grenzgebiete, Vol. 3. W.GERTLER (1973) Leipzig: Thieme. Pp. 1379–2253, illustrated. Price £13.60.

This is the third and final volume of the massive textbook by Wolfgang Gertler, formerly Director of the Dermatological Clinic of the Humboldt University of Berlin. Volumes 1 and 2 were reviewed in this journal in April 1971 and January 1973 respectively. This volume covers the mycoses, the reticuloses, tumours of the skin, cutaneous manifestations of systemic disease, stomatology, genito-pathology, the skin appendages and a miscellaneous collection of topics such as cosmetic dermatology, and history.

The somewhat complicated numerical classification and the telegraphic style will no longer deter those dermatologists who use the earlier volumes and have come to rely on them as a source of information and references. It is particularly as the latter that this work can be recommended to librarians, for the long bibliographies, which end each chapter, cover the German language literature most extensively. References to publications in other languages are relatively few and are sometimes incorrect. It is difficult too to discover on what basis references have been selected or excluded. For example, page 2165, headed General Literature, rightly lists Montagna's 'Advances in Biology of Skin', Volume 6, but fails to mention the other volumes in this outstanding series or the same author's indispensable 'Structure and Function of Skin'. In most chapters the text is reliable and up to date, but as in the above examples, chance appears to have determined what publications have come to the author's notice. The most recent reference to pityriasis versicolor is of 1955, and *Pityrosporum orbiculare* fails to find a place in the index. Further examples of such lacunae are easy to find, and probably reflect the still sadly inadequate medical and cultural contacts between East Germany and much of Western Europe and the United States. However, if the British or American reader bears in mind that the recent literature of their countries is sometimes partially neglected, he will find in this work a counterbalance to the frequent neglect of German publications by English speaking authors. The intending author of a 'comprehensive' review would do well to consult Gertler.

Despite these criticisms one can only congratulate Professor Gertler on bringing this monumental compilation to a successful conclusion. It deserves a place in every large department of dermatology.

ARTHUR ROOK

Epicutantestung. Einfuhrung in die Praxis. H.-J.BANDMANN and S.FREGERT (1973) Berlin: Springer. Pp. 100, 3 figures, numerous tables. Price £2.00.

This little book has been written by two members of the International Contact Dermatitis Research Group, and can be taken to represent the Group's combined views on patch testing. Of a size to fit any pocket or brief case, it gives practical details of patch testing and photo-patch testing, of how to make up one's own patch test materials, and exactly how and where to buy them commercially. In the second half of the book each of the common sensitizers is considered separately, including lists of obvious and obscure sources of the offending chemical.

The book is written in German but the lay-out is so clear that only a very modest command of the language is needed. It is understood that an English and other editions are forthcoming. No doubt every doctor, whether beginner or expert, concerned with investigating eczemas will then want to have this book ready to hand, not only in the library but more especially in the clinic.

R.H.CHAMPION

Speaking at Medical Meetings: a Practical Guide. JAMES CALNAN and ANDRAS BARABAS (1972) London: Heinemann. Pp. 111. Price £1.25.

And it is just that; a Very Practical Guide indeed. Soft-covered and small enough to slip into a pocket, it is the lecturer's *vade mecum.* I enjoyed reading it as much as the authors must have enjoyed writing (and illustrating) it. Only the captions are by courtesy of Shakespeare—and delightfully apt they are. Much has been written about the presentation of medical articles but little about the equally difficult art of verbal communication in medical meetings. The authors implied that, like acting, the technique is learnt, not inborn. Thus preparation and practice are essential. Some, of course, are better actors than others. I don't suppose that dermatologists differ much from the rest (though at least we have better scenery and can let our backcloths dazzle the eye when words fail and disaster threatens).

All the sins we have seen committed—and have committed ourselves—are spelt out in a text distinguished by its clarity and in a style deserving high praise. Tongue-in-cheek at times ?—well, perhaps; such accomplished professionals have to make it sound easy. The advice is always sensible and strictly practical. Almost every problem and form of meeting or confrontration is covered (though the authors sensibly baulk at the Organization of International Meetings). I did search in vain for instruction on enforced lecturing with a cold in full flood and on 'How to Deal with Foreigners'—as speakers or as audience. But the refined form of medical heckling known as 'questions' is most amusingly (and constructively) dealt with.

This cheap and very pleasantly produced Do-It-Yourself-Manual is really essential reading and could with advantage be presented not only to every junior by his Chief but—dare I say it—to every Chief by his staff.

D.S. WILKINSON

British Journal of Dermatology (1974) **90,** 593.

REPORT OF THE INTERNATIONAL COMMITTEE OF DERMATOLOGY

(Executive Committee of the International League of Dermatological Societies)

The International Committee of Dermatology met on 16 and 17 July 1973 in London, England, under the Presidency of Professor R.L.Baer, to discuss the form of future international congresses of dermatology and to begin the preparations for the XV International Congress of Dermatology which will take place on 16–22 October 1977 in Mexico City.

The following members of the International Committee were present: O.Braun-Falco, German Federal Republic; J.Civatte and R.Degos, France; J.Gay-Prieto, Spain; S.Jablonská, Poland; A. Padilha-Gonçalves, Brazil; F.Sagher, Israel; N.Thyresson, Sweden; H.J.Wallace, Great Britain; R.Winkelmann, U.S.A.; as well as the President and Secretary of the XV International Congress of Dermatology in Mexico City, A.Gonzalez Ochoa and L.Dominguez Soto, Mexico; and the President and Secretary of the last, XIVth International Congress of Dermatology in Venice/Padua, F.Flarer and F.Serri, Italy; and T.Yasuda, Japan, by invitation.

All participants were in agreement regarding the purposes which international congresses of dermatology must fulfil. They are useful only if they adhere to high scientific standards, present new and important data, and include a programme educational both for scientifically interested and for practising dermatologists. In addition, such congresses must provide opportunities for the participants, whether practising dermatologists or basic scientists, to enter into direct discussions with the authorities in various fields of science and practice. After detailed discussions of these problems, the following activities were decided upon for the XVth International Congress of Dermatology in Mexico City.

(1) Main themes will be presented daily between 9 a.m. and 1 p.m. in the large auditorium. The topics will be the newest developments of importance in the areas of clinical dermatology and dermatological aspects of basic science. The speakers will be invited. The presentations will last 30–40 min and will not be discussed.

(2) Symposia will deal with newer developments in clinical dermatology and dermatological basic sciences. About fifteen symposia, each with one particular topic, are planned. Some of the speakers will be invited while others will be those proposed by the national dermatological societies.

(3) Workshops will deal with circumscribed topics in the basic or clinical sciences in dermatology. Participation will be by invitation.

(4) Educational courses will serve the continued education of dermatologists in the scientific basis of practical dermatology. Each day, a particular topic will be covered between 2.30 and 6 p.m. The course director will be responsible for the planning of the entire course.

(5) Informal tea discussion groups will take place in the hotels between 5 or 5.30 and 6.30 or 7 p.m. They will be led by experts in particular fields. The discussion groups will provide closer contact between the practising dermatologists and the experts.

(6) Free communications will be included. However, a committee will be appointed which will review the abstracts submitted and decide whether the communications should be presented, read by title only or rejected.

(7) Patient demonstrations are particularly important at international congresses of dermatology and deserve greater attention than they have previously received. The form of patient demonstration (for example, by slide presentation, film, etc.) has not yet been determined.

To allow for optimal planning of the congress, the International Committee has formed sub-committees which will concern themselves with Case Demonstrations, Main Themes, Symposia, Educational

Courses and Workshops. The co-ordinators of these sub-committees will contact not only the national Dermatological Societies, but also the Society for Investigative Dermatology and the European Society for Dermatological Research, to ensure the most effective planning of the scientific programmes.

Professor Gonzalez Ochoa, the President, and Dr Dominguez Soto, the Secretary of the International Congress of Dermatology in Mexico City in 1977, discussed questions of organization and informed the Committee that the Mexican authorities have agreed to have the Congress take place on 16–22 October 1977.

There was extensive discussion of the question of publication of the congress proceedings. For a number of reasons the International Committee came to the conclusion that in place of a volume on transactions, a book of abstracts should be published at an early date before the congress. Transactions of international congresses now have only limited value and do not justify the very high cost of publication.

British Journal of Dermatology (1974) **90**, 595.

News and Notices

Third World Congress of the International Society of Tropical Dermatology

This Congress will be held in São Paulo from 1 to 5 September 1974, under the Presidency of Professor S.A.P.Sampaio. Details of the programme and other information may be obtained from the Secretary–Treasurer, Professor Raymundo M.Castro, M.D., Third World Congress of Tropical Dermatology, c/o Alcantara Machado Congressos S/C LTDA, Palacio das Convencoes, Parque Ahembi, CEP–02012, São Paulo–SP, Brazil.

Symposium on Collagen in Health and Disease

A Symposium on Collagen in Health and Disease will be held in the University of Bristol on 27 and 28 September 1974. All those who are interested should write for further information to the Rheumatology Research Secretary, University Department of Medicine, Bristol Royal Infirmary, Bristol BS2 8HW.

University of Cambridge School of Clinical Research and Postgraduate Medical Teaching

Medicine and Mycology

A course on common fungal infections will be held at Fitzwilliam College, Cambridge, on 25–27 September 1974. The course is intended for all clinicians and laboratory workers interested in diseases caused by fungi. It should prove especially valuable to dermatologists and clinicians working in the field of respiratory disease, leukaemia and reticuloses, and organ transplantation, but it is hoped that stomatologists, obstetricians and gynaecologists, paediatricians and all who use immunosuppressive therapy will find much that is relevant for them. For medical microbiologists the course emphasizes the clinical problems and the role of the laboratory in diagnosis and management. It is less concerned with the technical aspects of medical mycology and the identification of fungal species.

The lecturers will include: R.A.Cawson, G.P.Clein, R.R.Davies, D.B.Evans, E.G.V.Evans, Andrew Henderson, B.M.Herbertson, J.R.Hobbs, Rosalinde Hurley, David Kerridge, D.W.R.Mackenzie, M.F.Madelin, R.R.Marples, S.O.B.Roberts, Arthur Rook, M.E.Snell, and D.C.E.Speller.

Enrolment forms may be obtained from the Secretary, Cambridge University Medical School, Hills Road, Cambridge CB2 2QL.

APPOINTMENTS

Derbyshire Royal Infirmary

Dr Roger B.Allen has been appointed Consultant Dermatologist to the Derbyshire Royal Infirmary. He qualified from Bristol University in 1964 and became M.R.C.P.(U.K.) in 1970. He did his general medical training at the Royal Infirmary, Sheffield, and later became Registrar in Dermatology in Sheffield and Senior Registrar in Dermatology at the Royal Infirmary, Edinburgh. He has published a number of papers in general medicine and dermatology.

St John's Hospital for Diseases of the Skin, London

Dr Colin Andrew Ramsey has been appointed Consultant Dermatologist with an interest in Photobiology, to St John's Hospital for Diseases of the Skin, London. After qualifying M.B., B.S. at King's College Hospital Medical School in 1959, Dr Ramsey worked in general medicine, paediatrics and infectious diseases. He obtained the M.R.C.P. (London) in 1964 and started training in dermatology at The London Hospital. The year 1968–69 was spent as a Post-Doctoral Research Fellow at The University of Wisconsin Medical School where he obtained considerable experience in photobiology. He was then appointed Senior Registrar and Tutor at St John's Hospital for Diseases of the Skin, and, in 1971, Senior Lecturer in the Department of Photobiology at The Institute of Dermatology and Honorary Consultant Dermatologist to St John's Hospital during the tenure of a Medical Research Council grant. He proceeded to the M.D. in 1973. Dr Ramsey was appointed Consultant Dermatologist to Guy's Hospital, London, in January 1974. His principal publications have been on dermatological photobiology and on the physiology of the blood supply of the skin.

Mid-Antrim Group of Hospitals

Dr R.S.Matthews has been appointed Consultant Dermatologist to the Mid-Antrim Group of Hospitals, Northern Ireland. Dr Matthews qualified M.B., B.Ch., B.A.O. from Queens University Belfast in 1964, and became a Member of the Royal College of Physicians (Ireland) in 1967.

After short periods in physiology, medicine and paediatrics he became Senior Registrar in Dermatology in 1969. He did a period of research work on various immuno-fluorescence studies on warts and molluscum contagiosum.

Following this he worked in dermatology in Pretoria, South Africa, in Jerusalem, Israel, and in St John's Hospital for Diseases of the Skin, in London.

Newcastle University, Gateshead and District and North-West Durham Groups of Hospitals

Dr Michael George Cameron Dahl has been appointed Consultant Dermatologist to Newcastle University, Gateshead and District and North-West Durham Groups of Hospitals. Dr Dahl qualified M.B., B.Chir., 1965, from the Middlesex Hospital, London, and became M.R.C.P. (London) in 1968. He did his post-graduate work at the Middlesex Hospital and later the Cheltenham General Hospital before moving as Senior House Officer to St John's Hospital for Diseases of the Skin. In 1969 he became M.R.C. Junior Research Fellow and Honorary Registrar in the Department of Dermatology, Royal Victoria Infirmary, Newcastle, later becoming Senior Research Associate and Honorary Senior Registrar in that Department. His publications include several on liver damage due to methotrexate in patients with psoriasis.

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Textbook of Dermatology

Edited by

Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Robert Harold Champion M.A. M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D.(Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Niels Hjorth M.D.(Copenhagen)
William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
Francisco Kerdel-Vegas M.D.(Caracas) M.SC.(N.Y.)
Gunnar Lumbolt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
Stephen Owen Bellamy Roberts M.A. M.B. B.CHIR.(Cantab.) M.R.C.P.(Lond.)
Arthur James Rook M.D.(Cantab.) F.R.C.P.(Lond.)
Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
Peter Derrick Samman M.A. M.D.(Cantab.) F.R.C.P.(Lond.)
Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
Ian Bruce Sneddon M.B. CH.B.(Sheffield) F.R.C.P.(Lond.)
Darrell Sheldon Wilkinson M.D.(Lond.) F.R.C.P.(Lond.)
Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Leonard Beard M.A.(Cantab.) A.I.I.P. F.R.P.S.

Second Edition, 1972. 2236 pages, 1100 illustrations.
Two volumes in slip case, £32.50

Clinical Tropical Dermatology
Edited by Orlando Canizares M.D. F.A.C.P. Autumn 1974. 700 pages, 272 illustrations (40 colour). About £10.00

Lecture Notes on Dermatology
Bethel Solomons M.A. M.D. F.R.C.P.I. *Third Edition*, 1973. 268 pages, 107 illustrations (37 colour). Paper, £2.25

Manual of Dermatologic Syndromes
Thomas Butterworth A.B. M.D. MED.SC.M. *Second Edition*, 1972. 104 pages. Lippincott, £1.50

Cutaneous Lesions of the Lower Extremities
M.H. Samitz M.D. M.SC.(Med.) and Alan S. Dana Jr M.D. 1972. 210 pages, 132 colour illustrations. Lippincott, £14.00

Blackwell Scientific Publications

Oxford London Edinburgh Melbourne

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also quarterly reviews of recent advances in contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) $(10^{-3}$ g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) $(10^{-6}$ g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) $(10^{-9}$ g)	ng	millimetre(s)	mm	percent	%
picogram(s) $(10^{-12}$ g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Rook, A., Wilkinson, D.S. & Ebling, F.J.G. (1968) *Textbook of Dermatology*, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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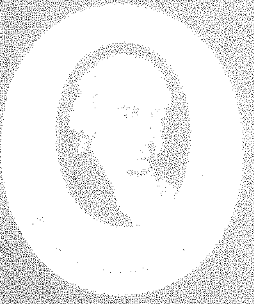

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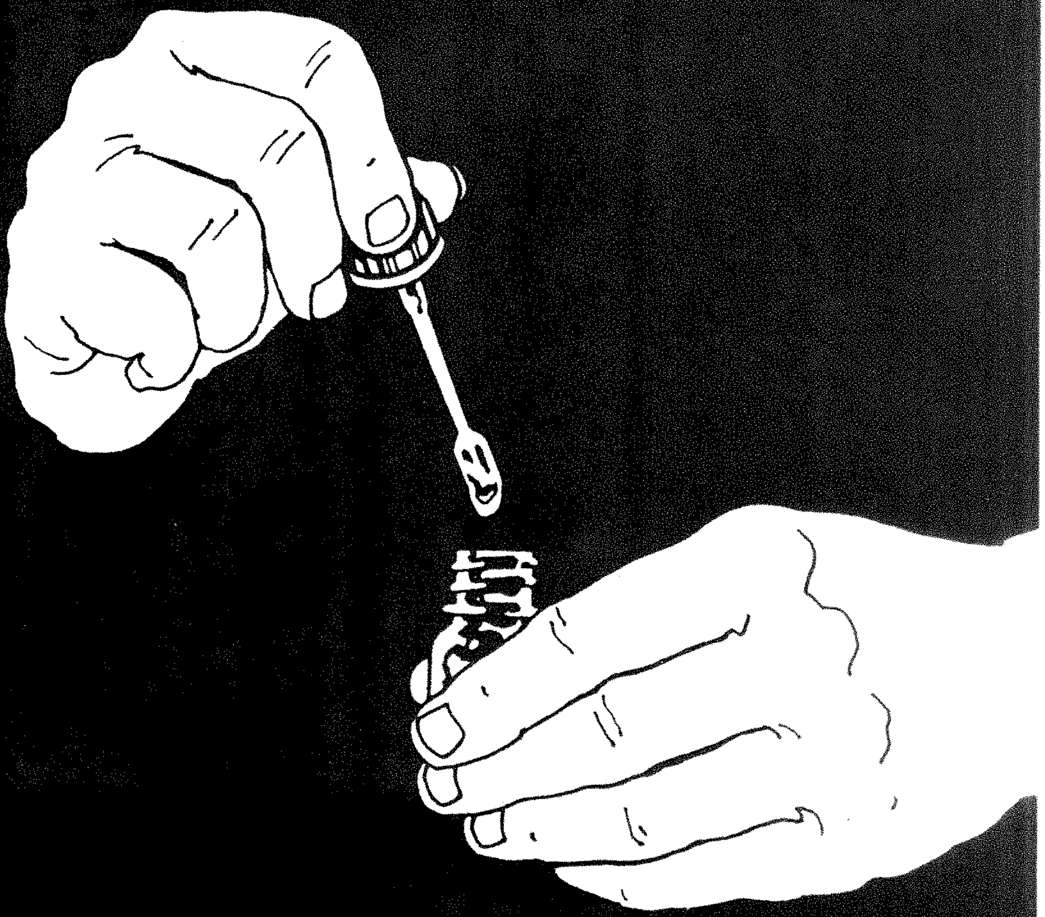

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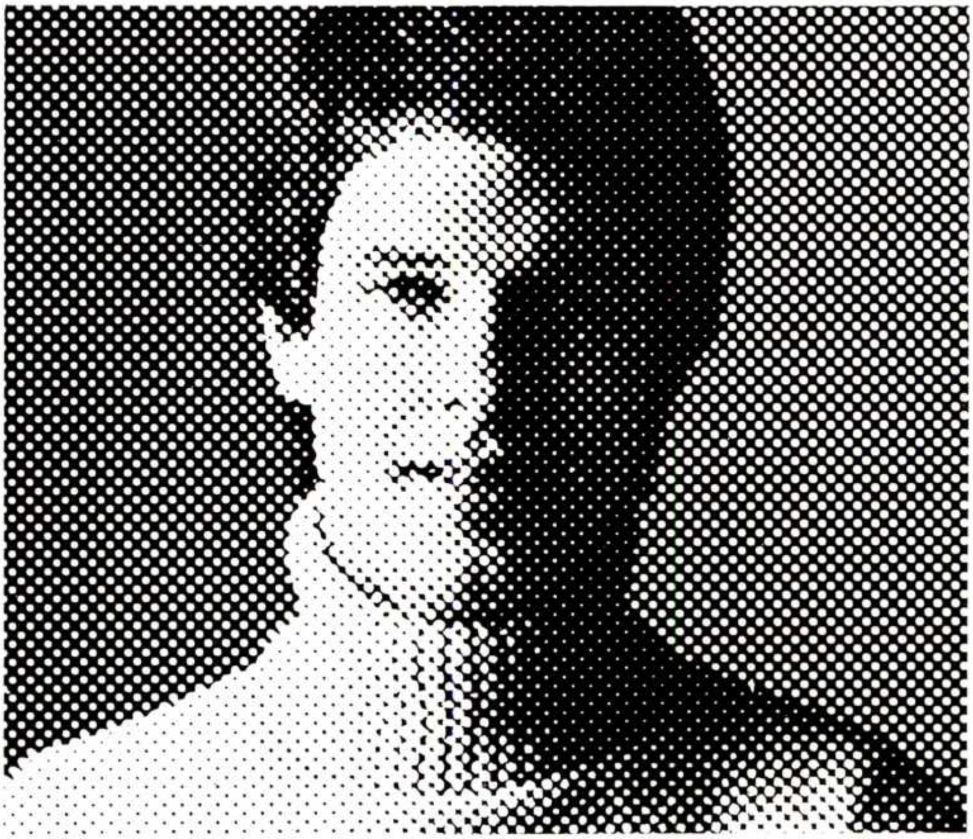

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1 *Indian.J.Dermatol.Venereol.*, **36**, 100, 1970
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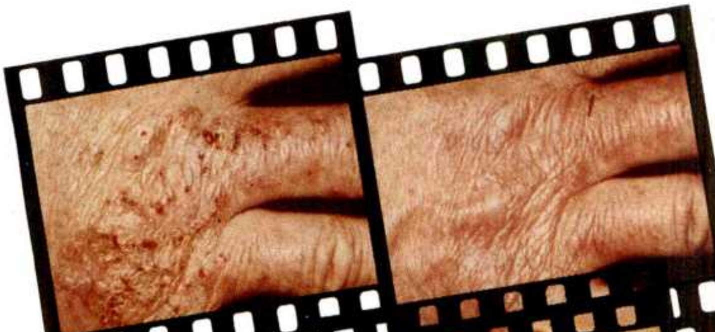

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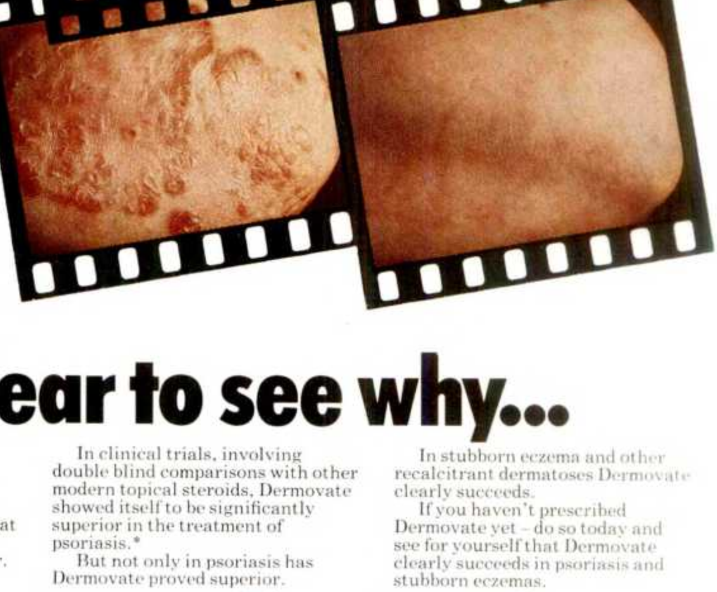

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*British Journal of Dermatology, 1974, **90** (In Press)
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THE DIAGNOSIS AND TREATMENT OF FUNGAL INFECTIONS: A Symposium. *Compiled and edited by* **Harry M. Robinson, Jr.,** *Univ. of Maryland. (38 Contributors)* The experienced dermatologist, medical mycologist and internist will find this text valuable. It covers recent developments in microbiology, laboratory diagnosis, histopathology, immunology and recent experimental work. Therapeutic procedures described represent the latest approved methods and articles include various subjects concerning the pathology of both superficial and deep mycotic infections. Many of the authors who have contributed to this text participated actively in the original studies which made these advances possible. *'74, 580 pp., 128 il. (18 in full color), 45 tables, $29.50*

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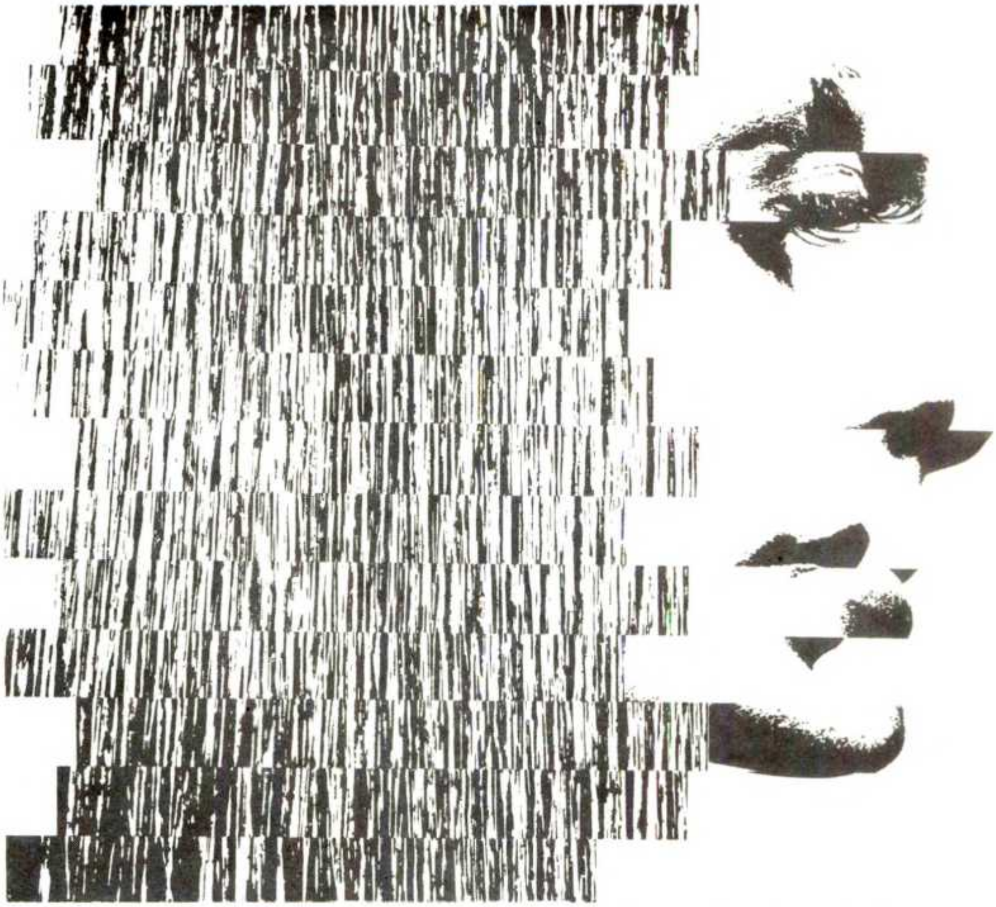

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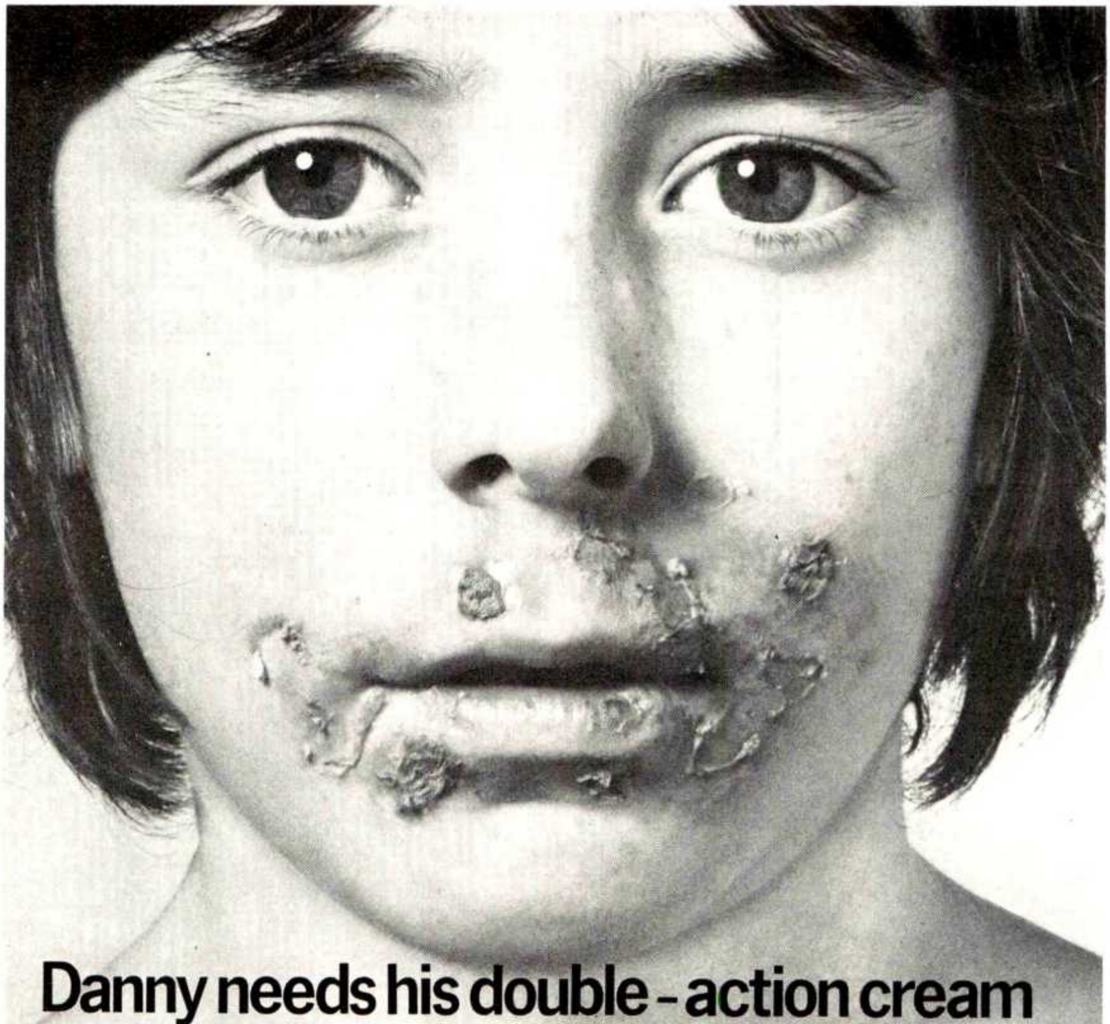

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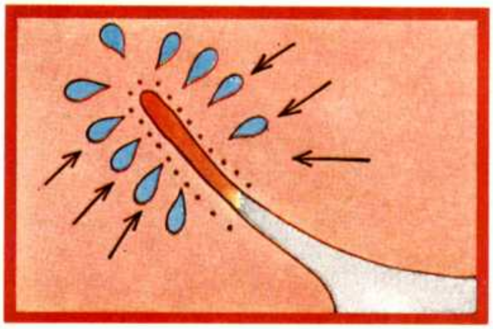

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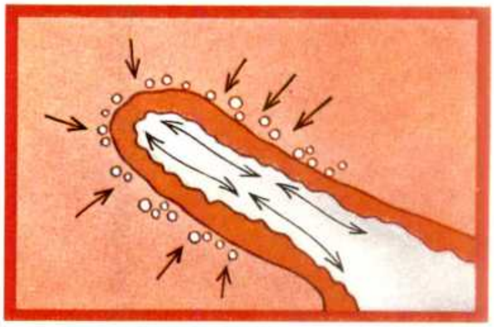

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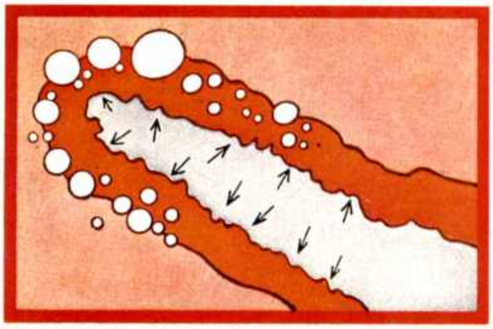

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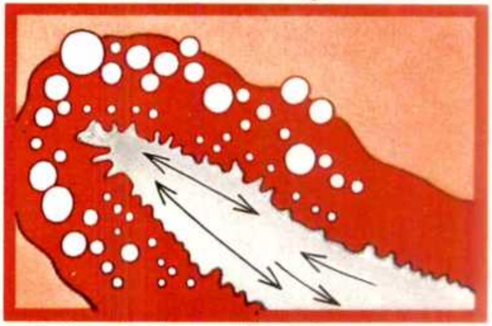

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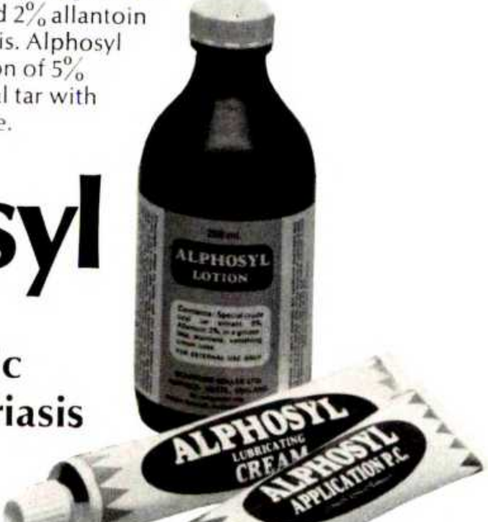

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1. *Practitioner* (1970) **205:** 452.
2. *Brit. J. Derm.* (1968) **80:** 791.
3. *Practitioner* (1970) **204:** 625.
4. *Brit. J. Derm.* (1965) **77:** 163
5. *Brit. med. J.* (1967) **4:** 535

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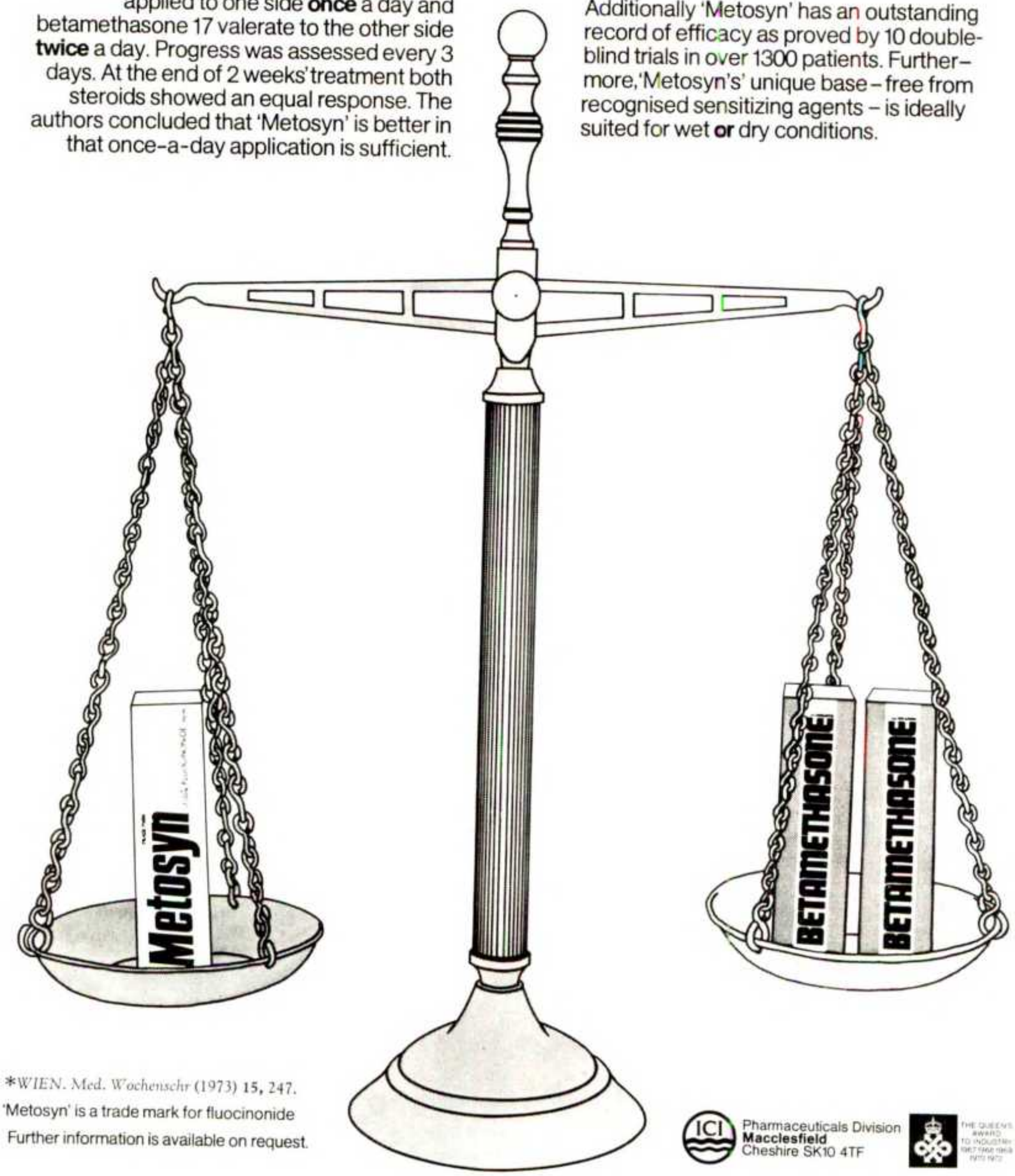

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1. Almeyda, J. and Burt, B.W., *Brit. J. Derm.* (To be published) 2. Harries, E. H. L. and Jacoby, R. H. (To be published)
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British Journal of Dermatology (1974) **90**, 597.

Clinical and Laboratory Investigations

Immunoglobulin- and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema)*

R.H.CORMANE,† S.HUSZ‡ AND F.HAMERLINCK†

† Department of Dermatology, University of Amsterdam, Binnengasthuis, Amsterdam, The Netherlands, and
‡ Department of Dermatology (Chairman: M. Simon), University of Szeged, Hungary

Accepted for publication 28 September 1973

SUMMARY

The distribution pattern of immunoglobulin- or complement-bearing lymphocytes in healthy controls and in patients with contact dermatitis and with atopic dermatitis has been investigated. A significant predominance of IgD- and C3-bearing lymphocytes was found in ten patients with contact dermatitis. In atopic dermatitis patients the number of IgE- and to a lesser extent of C3-bearing lymphocytes was increased. The results indicate induction or stimulation of two distinct classes of immunologically competent lymphoid cell lines, one in contact and the other in atopic dermatitis. The increased number of B-cells in dermatitis indicates a decreased T-cell population and suggests a deficiency or disturbance of the recirculation of this population.

Immunofluorescence staining can be very useful to detect immunoglobulin (Ig) on the surface of living lymphoid cells. Little has been reported on the occurrence of these cells in human skin (Ueki, 1967; Baart de la Faille-Kuyper, 1969; Lai a Fat, Cormane & van Furth, 1974).

Direct immunofluorescence (DIF) staining of cryostat sections, taken from positive patch tests on ten patients with contact dermatitis caused by various antigens, using fluorescein-conjugated monospecific antisera to the five main heavy-chain classes of Ig and to complement, revealed the presence of lymphoid cells with membrane-bound Ig and complement (C3) in the dermis. This observation induced us to study the distribution pattern of Ig- and C3-bearing lymphocytes in the peripheral blood of patients with contact dermatitis and atopic dermatitis (Cormane, Husz & Hamerlinck, 1973).

MATERIALS AND METHODS

Peripheral blood lymphocytes

Ten patients with contact and ten with atopic dermatitis were the source of human lymphocytes.

---

* Based on a paper presented at the third annual meeting of the European Society for Dermatological Research, Amsterdam, The Netherlands, April 1973.

Normal healthy department members (ten) served as controls. The various antigens to which the patients with contact dermatitis were sensitive are listed in Table 1.

Lymphocytic separation

Lymphocytic separation was carried out according to the method of Böyum (1968). 20 ml of peripheral blood was collected by venepuncture in Erlenmeyer bats containing fifteen glass perles, and shaken for 10 min. After defibrination, 60 ml isotonic NaCl solution was added. Of this suspension 8 ml was added to 3 ml Ficoll Isopaque solution. The latter suspension was centrifuged at 400 **g** for 20 min. The resultant lymphocyte-rich layer between the Ficoll Isopaque and the supernatant was aspirated and washed three times in isotonic NaCl solution, and after each washing was lightly centrifuged at 200 **g**. The cells thus obtained were resuspended in isotonic NaCl solution, to give about 5 million cells per ml, composed of about 90–95% lymphocytes and 5–10% monocytes and neutrophils. For the separation, only thoroughly cleaned and siliconized glassware was used.

TABLE 1. Patients with contact dermatitis

Patient no.	Positive patch tests after 48 h
1	Hydroquinone 5% aq., benzoylperoxide 10% vas.
2	Nickel sulphate 2·5% vas.
3	Cobalt chloride 1% vas., balsam of Peru 25% vas.
4	Tetramethylthiuram disulphide 2% vas., mercaptobenzothiazole 2% vas. Formaldehyde 2% aq.
5	Nickel sulphate 2·5% vas.
6	Potassium dichromate 0·5% vas.
7	Cobalt chloride 1% vas.
8	Cobalt chloride 1% vas.
9	Hydroquinone 5% aq., stannous chloride 0·5% aq.
10	Mercaptobenzothiazole 2% vas.

Immunofluorescence staining and examination

The chemical and immunochemical data of the conjugates used are listed in Table 2.

Direct staining of cells in suspension was carried out by suspending 2×10^5 washed lymphocytes in 0·25 ml of the 1:32 diluted conjugate; this was then incubated at 37° C for 30 min. After incubation, the cells were washed for 15 min in isotonic NaCl solution and resuspended in 0·2 ml of the same solution.

For the smear technique, the object slides were initially cleaned in 96% ethanol and then passed through a gas flame. One drop of the lymphocyte suspension was sufficient for a thin cell smear. The smears were made very cautiously. No fixation procedures were applied. After drying at room temperature for about 15 min, the smears were washed in phosphate buffer pH 7·2 and subsequently incubated for 30 min with the conjugate. Before mounting with Difco mounting fluid the slides were washed twice in the buffer for 15 min.

Immunofluorescence examination was carried out on the suspension and on thin smears. In each case the percentages of Ig- and C3-positive lymphocytes were determined by examination of the microscope field alternately in blue narrow-band (480–490 nm) epi-illumination for fluorescence and in conventional light. Fluorescing cells, in most instances, showed a homogeneous or coarsely

TABLE 2. Characteristics of FTC-protein conjugates

No.	Conjugates*	Batch number	FTC content (μg/ml)	Protein content (mg/ml)	F/P ratio (μg/mg)	Molar ratio F/P × 0·411	Antibody unitage	Dilution used for staining
1	Rabbit anti-human IgG/FTC	871†	17·3	12·6	1·37	0·56	4	1/32
2	Goat anti-human IgA/FTC	6-171†	31·04	32·8	0·94	0·38	1	1/32
3	Goat anti-human IgM/FTC	7-171†	88	51·9	1·52	0·62	4	1/32
4	Rabbit anti-human IgD/FTC	KH20-5-A1‡	96	16·4	5·1	2·1	4	1/32
5	Rabbit anti-human IgE/FTC	KH25-4-P1‡	60·6	11·4	5·3	—	—	1/32
6	Rabbit anti-human β_1A/FTC	KH41-10-P1‡	92·4	12·4	7·4	3·0	4	1/32
7	Rabbit anti-swine	2-969†	18·3	15·6	1·17	—	—	1/32

* Antisera labelled with FITC.

† Nordic Immunological Laboratories, Tilburg, The Netherlands.

‡ The unconjugated antisera were obtained from Central Laboratory of Red Cross Blood Transfusion Services, Amsterdam, and were labelled in our laboratory by S. S. Asghar, Ph.D.

—, not studied.

FTC content of conjugates was determined by the method of McKinney, Pearce & Spillane (1966) using fluorescein diacetate (FDA) as reference standard. Protein content was measured by the method of Lowry *et al.* (1951). Antibody unitage of conjugates was determined in a standard gel diffusion test using 1 mg/ml respective antigens, except in case of FTC/anti β_1A conjugate where normal serum was used as a source of antigen, against doubling dilutions of conjugates as described by Beutner, Sepulveda & Barnett (1968). A 1 mg/ml solution of human IgG, IgM, IgA and IgD contained 6·6, 129, 30·5 and 100 antigen units respectively in relation to international reference serum.

Staining properties and specificity of the class-specific conjugates (1–5) were tested in indirect IF in normal and monoclonal human bone marrow plasmacytes.

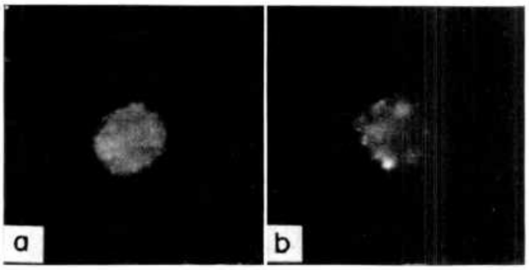

FIGURE 1. Peripheral blood lymphocytes stained in suspension with RAH-IgD/FTC (dilution 1:32). (a) Lymphocyte with a homogeneous, coarsely grained, staining pattern (assessed), (b) lymphocyte with a sectoral staining pattern (ignored). Blue narrow band excitation, objective × 50, water immersion, ocular × 6.

grained staining (Fig. 1a). The presence of a sectoral pattern was seen in few cells (Fig. 1b). The latter cells were ignored in the assessment. Trypan blue viability of the cell preparations utilized was greater than 97% and was performed before IF staining.

Specificity tests

Fluorescein conjugated swine anti-rabbit antiserum (SwAR/FTC) failed to stain the smear cells. The addition of normal rabbit serum to the cell suspensions did not reduce the numbers of cells staining.

Unconjugated antiserum specifically inhibited staining due to its own conjugated counterpart but did not inhibit staining due to a conjugated antiserum directed against a different class of immunoglobulin.

Microscopy

A Leitz Orthoplan Microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator were used. FTC fluorescence was detected with the filter combination BG 38 (4 mm), KP 490 (2 mm), GG 475 (2 mm) and secondary filter OG 515 (3 mm). In all cases the examination was performed with Leitz water immersion objectives (×50 and ×90).

In each case, the percentage of Ig-positive lymphocytes (lymphocytes with characteristic membrane fluorescence) was estimated by examination of the same microscopic field alternately in specific illumination for fluorescence and in conventional light. Microphotographs were taken with an Orthomat camera using Anscochrome 500 daylight film.

RESULTS

Both the smear technique and the counting chamber method (in suspension) have their advantages and disadvantages. The calculation of the percentages of cell populations is much more accurate in counting chambers than in smears; however, the smears can be stored at –90°C for at least 1 week, whereas cells in suspension have to be stained and counted on the day the blood is taken. Specificity tests can be carried out more easily on smears. Very important is the fact that staining for complement factors can be done only on smears because the addition to cell suspensions of FITC-labelled anti-complement antisera causes agglutination of the cells.

TABLE 3. Distribution pattern of Ig-bearing lymphocytes in healthy controls

Authors	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%
Bert *et al.* (1968)	10–20	10–20	10–20	—*	—	10–20
Verma *et al.* (1971)	—	—	—	—	—	23·4
Papamichail *et al.* (1971)	—	—	—	—	—	20–30
Papamichail *et al.* (1972)	16	6	7	—	—	16
Fröland & Natvig (1972)	7·9	0†	9·7	—	—	14·3
van Boxel *et al.* (1972)	—	—	—	2·7	—	16
Piessens *et al.* (1972)	11	8	11	6	1	—
Aiuti & Wigzell (1973)	7·9	4·6	9·2	—	—	—
Cormane *et al.* (1973)	4·1	1·2	5	3·5	2	15·8

* —, not studied.
† 0, not demonstrated.

In the ten controls the values for IgD- and IgE-positivity of lymphocytes were in fair accordance with those reported by van Boxel *et al.* (1972) and Piessens *et al.* (1972). The percentages of IgG-, IgA-, IgM- and total Ig-bearing lymphocytes were lower than generally mentioned in the literature

(Table 3). This discrepancy is mainly due to the fact that in our study only homogeneously stained cells were counted.

It is of note that in the ten controls complement (C3)-bearing lymphocytes, with a mean of 6·2% and a range of 3–8%, were also present (Table 4).

TABLE 4. Healthy controls

Control no.	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%	C3%
1	2	0	8	6	2	18	7
2	2	0·5	1	1·5	1	6	3
3	7	2	8	8	3	28	8
4	3	1	6	4	5	19	6
5	3	0·5	3	3·5	1·5	11 5	5
6	2·5	2	6	3	1	14·5	3
7	3·5	1 5	3	1·5	2	11 5	8
8	4	1	2	2·5	2	11·5	7
9	8	2	7	2	1	20·0	8
10	6	2	6	2·5	2	18·5	7
Range	2–8	0–2	1–8	1·5–8	1–5	6–28	3–8
Mean value	4·1	1 3	5·0	3·5	2·5	15·49	6·2

TABLE 5. Patients with allergic contact dermatitis

Patient no.	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%	C3%
1	2	1	8	54	1	66	68
2	3	0	3	30	0	36	40
3	3	5	4	20	4	36	15
4	12	2	8	65	—*	87	52
5	3	0	3	19	3	28	18
6	10	0·5	3	32	10	55·5	19
7	17	5	13	36	14	85	25
8	4·5	2	3·5	28	2	40	16
9	2	0	2	13·5	2	19·5	7
10	4	4	8	62	4	82	65
Range	2–17	0–5	2–13	13 5–65	0–14	19·5–87	7–68
Mean value	6·1	2·0	5·6	40	4·4	53·5	32·5

* —, not studied.

In all patients (ten) with contact dermatitis IgD was invariably found to be the dominant class on lymphocytes, with a mean of 35·9% and a range of 13·5–65% (Table 5, Fig. 2). The mean percentage (32·5%) and range (7–68%) of lymphocytes with C3 on their membranes were about similar to those given for IgD-positive lymphocytes. In some patients (nos. 1 and 4) more than 75% of the Ig- and C3-bearing lymphocytes consisted of cells with surface-bound IgD and C3.

In atopic patients (ten) IgE-bearing lymphocytes predominated in the peripheral blood, with a mean of 20·5% and a range of 14–31%; the mean of C3-bearing lymphocytes was 12·6% with a range of 6–33% (Table 6, Fig. 3).

TABLE 6. Patients with atopic dermatitis

Patient no.	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%	C3%
1	2	8	5	4·5	18	37·5	7·5
2	2·5	3	2·5	4	15·5	27·5	11
3	12	0·5	2	4	17	35·5	13
4	4	1	3	5	16	29	10
5	4·5	1·5	4·5	2	14	26·5	10
6	3	1	8	7	25	44	19
7	8	2	4	4	21	39	9
8	2	0·5	2	8	31	43·5	6
9	5	2·5	4·5	10·5	23	45·5	33
10	6	3	7	8·5	24·5	49	7·5
Range	2–12	0·5–8	2–8	2–10·5	14–31	26·5–49	6–33
Mean value	4·9	2·3	4·3	5·8	20·5	37·7	12·6

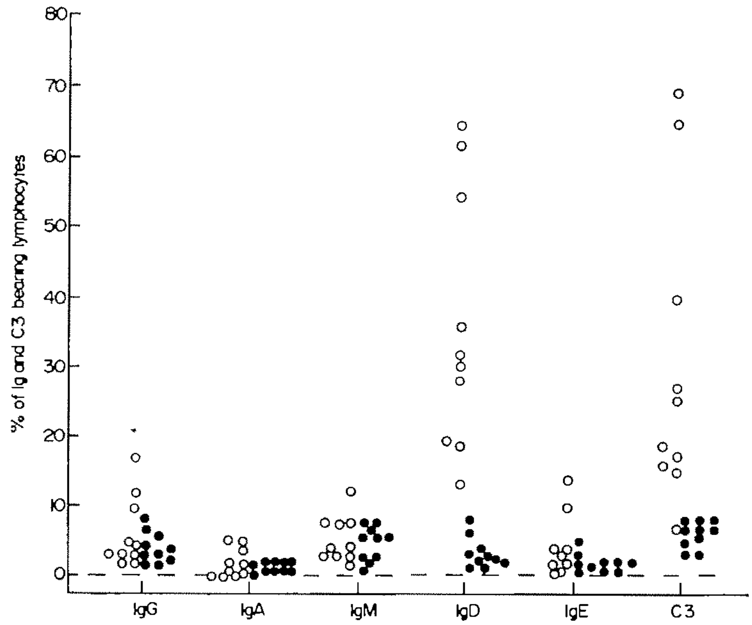

FIGURE 2. Distribution pattern of Ig and C3 bearing lymphocytes in patients with contact dermatitis.

On the cryostat sections the lymphocytes carrying Ig determinants and C3 on their membranes occurred scattered throughout the dermis in both forms of dermatitis.

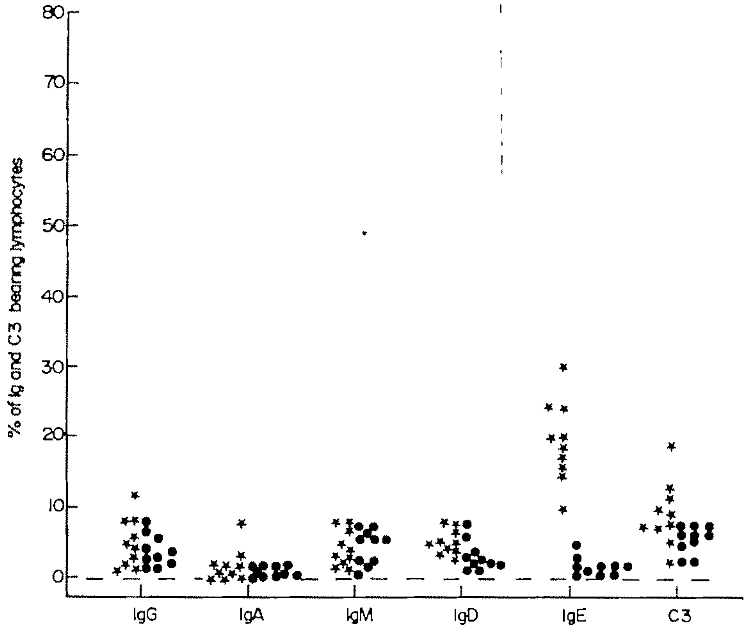

FIGURE 3. Distribution pattern of Ig- and C3-bearing lymphocytes in patients with atopic dermatitis.

DISCUSSION

From this study it appears that contact allergy can be distinguished from atopic dermatitis by means of the IF study of the distribution pattern of Ig-coated peripheral lymphocytes. In allergic contact dermatitis those peripheral lymphocytes bearing IgD predominate; whereas those coated with IgE predominate in atopic dermatitis.

The impression was gained that at least in contact dermatitis most of the IgD-positive lymphocytes are concomitantly bearing C3 on their membranes. This is difficult to explain because IgD does not fix complement. In atopic dermatitis IgE and C3 do not always occur concurrently. Investigations are in progress to elucidate this problem. It is of note that neither class of antibody has complement-bearing capacities.

The possibility of absorption due to probable high levels of IgD could be excluded by adding IgD to the lymphocyte suspensions of healthy controls. After incubation for 1 h at 37° C and subsequent washings, a normal IgD distribution profile was obtained, indicating that IgD is not cytophilic.

Genetic predisposition plays some role in contact dermatitis but a very much greater one in atopic dermatitis. This aspect may well reflect the predominance of special clones of lymphocytes in both types.

Long-term studies of both types of dermatitis have not yet been carried out by us. It seems that the predominance of the respective classes of Ig occurs independently of the severity and duration of the allergy. This preliminary observation may indicate that the predominance of a certain class of Ig may represent a stimulation of B-cells when the T-cell population is deficient or its recirculation is disturbed (Jacobsson & Blomgren, 1973).

In 'eczema hybrids', i.e. patients with concomitant atopic and contact dermatitis, we found an abundance of both IgD- and IgE-bearing lymphocytes (to be published).

The finding of IgE-bearing peripheral lymphocytes in atopic dermatitis is in accordance with the observations of Lobitz, Honeyman & Winkler (1972) who found IgE-coated small lymphocytes in the peripheral blood of two patients with severe atopic dermatitis.

It seems very probable that the IgD- and IgE-coated lymphocytes release a dermatitis-producing factor after contact with a complete antigen and that because they carry antigen receptors these cells play an important role in the pathogenesis of the eczematous lesions.

The tissue distribution pattern of the Ig- and C3-bearing lymphocytes favours the view that these cells are recruited from the bloodstream and are actively involved in the eczematous state.

In the evaluation of these findings it is interesting also to know whether the Ig- and C3-bearing lymphocytes have actually synthesized these molecules. Very recent findings, mainly stemming from animal experiments, indicate that membrane-bound Ig and C3 are markers of the bone marrow-derived 'B' cells, which are thought to be precursors of plasma cells (Dukor, Bianco & Nussenzweig, 1970). The ultrastructural studies of Papamichail, Brown & Holborow (1971) showed that these cells lack the rough endoplasmic reticulum associated with the ability to secrete immunoglobulins. It is well known that immunoglobulins are produced by plasma cells and lymphocytes (Gowans & McGregor, 1965; van Furth, Schuit & Hijmans, 1966a, b, c; Chessin *et al.*, 1968; van Furth, 1969; Hijmans, Schuit & Hulsing-Hesselink, 1971; Hijmans & Schuit, 1972) and not by other types of cells. From our study it is conceivable that B-lymphocytes also secrete complement factors.

Perhaps the most significant finding of our study is the induction of different lymphoid cell lines in contact and in atopic dermatitis. Most probably these cell lines are directed against the antigens involved. If the surface Ig serves as a receptor for an antigen to allow the initiation of antibody formation, the question arises as to the function of those cells which do not contain Ig on their surface. They may represent T-cells and cells whose function has nothing to do with that of Ig-bearing cells.

Our results are not unexpected since by the clonal selection theory the genetically selected lymphocytes would be present at all times, ready to react with antigen. On the other hand it is also quite possible that the Ig- and C3-coated lymphocytes are cells that have already reacted at least once with antibody, i.e. they are 'memory' cells.

Whether the IgD- and IgE-positive cells are actually in the slow process of Ig synthesis or whether they are truly in a resting stage that may last a long time, following an initial synthesis, is one of the major areas to be investigated.

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Ultrastructural studies on two cases of porokeratosis of Mibelli

PATRICIA R.MANN, D.F.CORT, E.A FAIRBURN AND A.ABDEL-AZIZ*

MRC Unit for Research on the Experimental Pathology of the Skin, The Medical School,
The University, Birmingham 15, and the United Birmingham Hospitals

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SUMMARY

The ultrastructural features of two cases of porokeratosis of Mibelli are described. They demonstrate that the basophilic granular material in the cornoid lamella consists of whole degenerate cells with pyknotic nuclei. The dermis contains clumps of a finely fibrillar material and some collagen, and many fibroblasts show degenerative changes.

Porokeratosis of Mibelli is a rare inherited skin disease which, in a significant number of cases, has been associated with the development of squamous carcinomata. The distinguishing histological feature of the disease is the cornoid lamella, a keratinous plug containing a column of basophilic granular material and below which the granular layer is absent. The nature and origin of this keratotic material have been much disputed, but it is now accepted from histological and histochemical studies that the disease does not originate in the sweat ducts, as originally postulated by Mibelli (1893). We have investigated the biopsy material from two cases of this condition, with special reference to the cornoid lamella, using electron microscopy.

The diagnosis of both cases was established clinically and the histology was unequivocal. Both cases have been reported previously, with emphasis on the pre-malignant nature of the disease (cases 1 and 2 of Cort & Abdel-Aziz, 1972) and cytogenetic studies on biopsies from these patients demonstrated chromosomal instability of fibroblasts in tissue culture (cases 1 and 3 of Taylor, Harnden & Fairburn, 1973).

Case 1

A Caucasoid male developed a small non-irritant eruption on the left leg at the age of 2 years. Over the next 10 years the lesions extended to involve the entire limb, with parts of the buttock, lower abdomen and loin. Early in adult life the upper arm became involved, but the process remained strictly localized to the left side of the body. Squamous cell carcinomata developed in some of the porokeratotic lesions. No family history of significance could be elicited.

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* Present address: Department of Dermatology and Venereology, Al-Azhar University, Cairo, Egypt.

Case 2

A Caucasoid female developed an asymptomatic eruption on both legs at the age of 2 years. Over the next 15 years the lesions extended to involve the arms as well. Within these areas, the process became more widespread and florid during her only pregnancy at the age of 30. The patient was aged 59 when this study was undertaken, and at this time involvement of the nails was also noted and squamous cell carcinomata were present in some of the lesions. There was no relevant family history.

METHODS

Biopsies were taken for both light and electron microscopy in such a manner as to ensure inclusion of the peripheral horny ridge of a lesion. Specimens were taken under plain lignocaine local anaesthesia and were immediately immersed in ice cold 5% glutaraldehyde in cacodylate buffer. Each piece was cut into cubes of approximately 1 mm^3. Fixation in glutaraldehyde for about 3 h was followed by washing overnight in several changes of cacodylate buffer before post-fixation in 2% osmium tetroxide in cacodylate buffer. The specimens were dehydrated in graded concentrations of ethanol and embedded in Epon. Survey 1 μm sections were cut and stained with toluidine blue. This enabled positive identification of the cornoid lamella to be made by light microscopy before ultrathin sections were cut. Where it was desired to examine the cornoid lamella, the block was trimmed so that only the cornoid lamella and a very small amount of adjacent epidermis were contained in the ultrathin sections. For comparative purposes, sections were also cut from blocks which appeared normal by light microscopy. All sections were collected on uncoated copper grids and stained with saturated methanolic uranyl acetate.

RESULTS

The histological findings were similar in both cases and were typical of the disease. The cornoid lamella was clearly seen at the periphery of the lesions. It consisted of a zone of altered keratin in the centre of which was a column of basophilic granular material and beneath which the granular layer was absent (Fig. 1). Epidermal appendages, e.g. sweat glands and hair follicles, were occasionally seen in relation to cornoid lamellae, which is to be expected in a gradually extending lesion. The central atrophic zone of the porokeratotic lesions showed hyperkeratosis, thin granular and Malpighian layers and effacement of the rete ridges. The dermis showed a chronic inflammatory infiltrate consisting of lymphocytes, histiocytes and plasma cells.

Ultrastructurally, the epidermis outside the area of the cornoid lamella showed few abnormalities, but there were some parts of the basal lamina which showed extensive replication (Fig. 2), and in places the basal layer was oedematous with widening of the intercellular spaces and attenuation of the desmosome–tonofilament complexes (Fig. 3). Melanocytes and Langerhans' cells were normal in appearance and distribution, as were the keratinocytes of the Malpighian layer. In this area the granular layer was present and was normal.

Below the cornoid lamella the same patchy changes were seen in the basal and Malpighian layers. The granular layer, however, was very inconspicuous with only very small deposits of keratohyalin. Within this defective granular layer were many dead and dying cells (Fig. 4). In some, the degeneration of cell organelles was associated with the formation of keratin-like material at the periphery of the cell. Subcellular organelles were almost always impossible to identify positively once the degeneration had started, indicating that it is a fairly rapid process. The cells became heavily vacuolated with cytoplasmic 'strands' between the nucleus and the cell membrane (Fig. 4). The vacuoles often contained dense granules (Figs. 4 and 5), and the nuclei of some of these cells were pyknotic. Many of the cells that

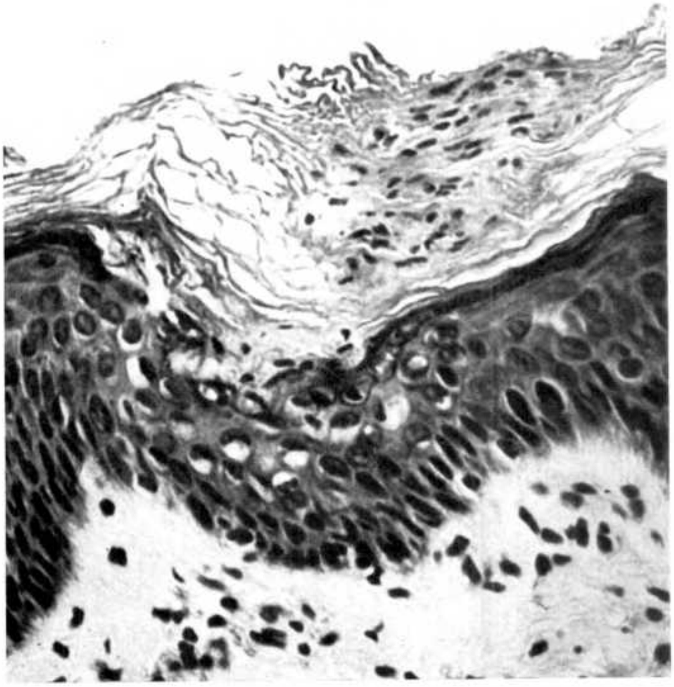

FIGURE 1. A typical cornoid lamella showing the basophilic granular material in the stratum corneum and the absence of a granular layer beneath this. Case 1 (H & E, × 120).

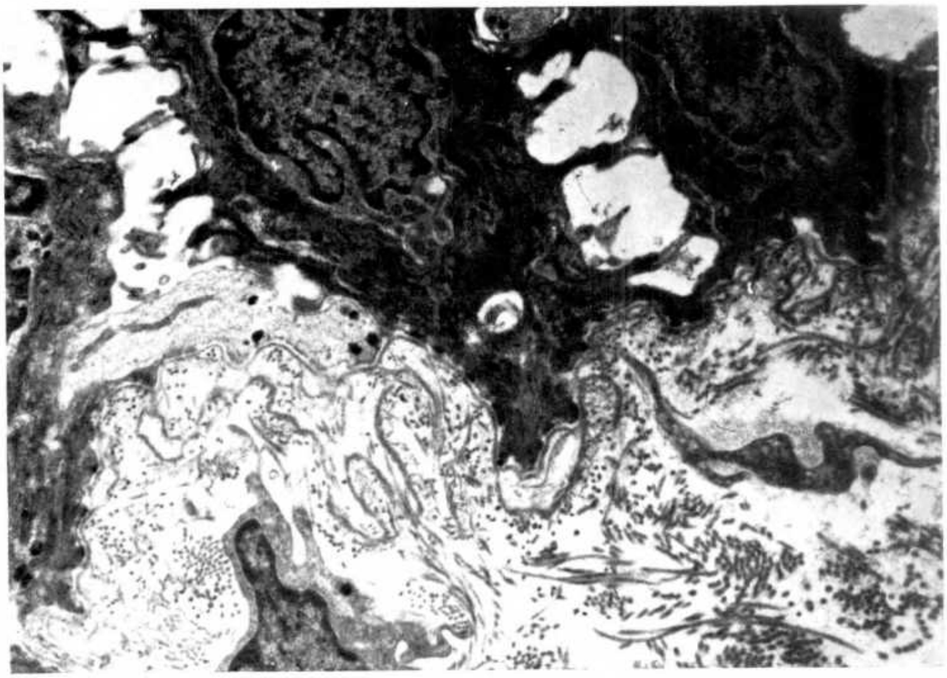

FIGURE 2. Dermo–epidermal junction showing basal laminar replication but normal keratinocytes and melanocytes. Case 2 (× 6400).

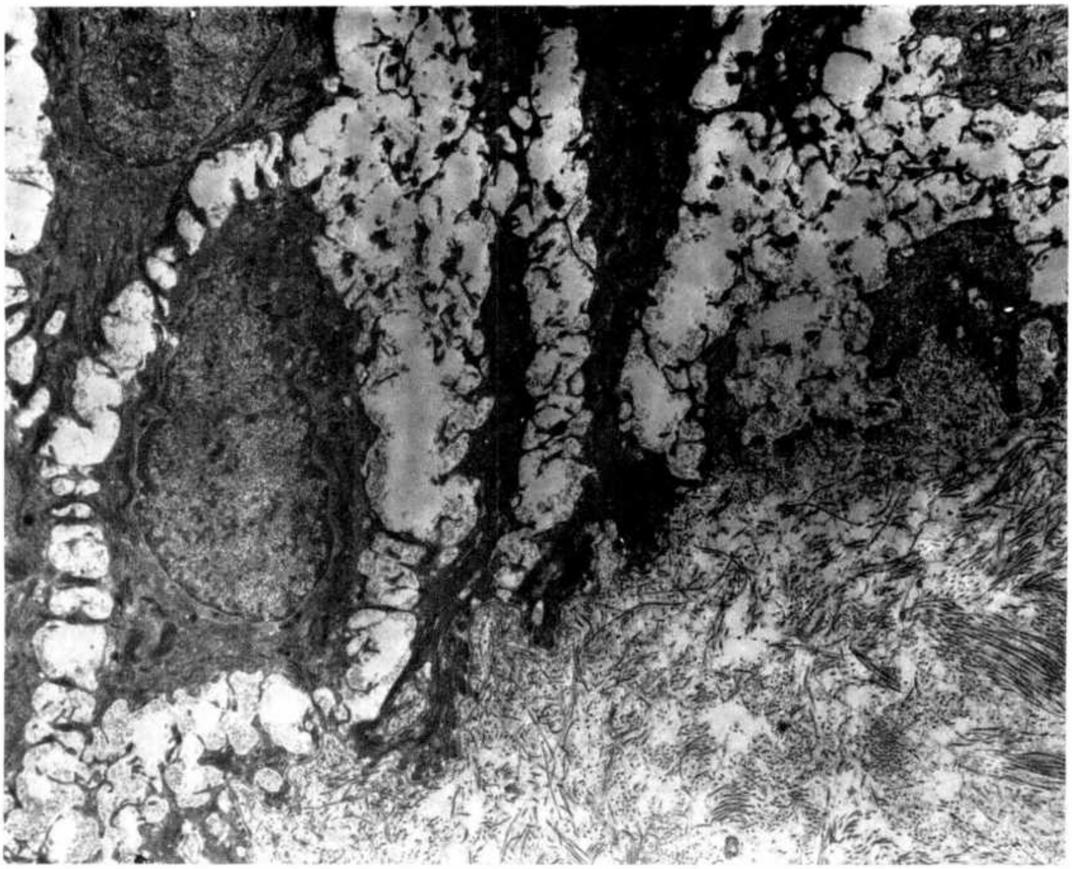

FIGURE 3. Oedematous basal layer of the epidermis with wide separation of the cells and a serous exudate in the intercellular space. Case 1 (×4400).

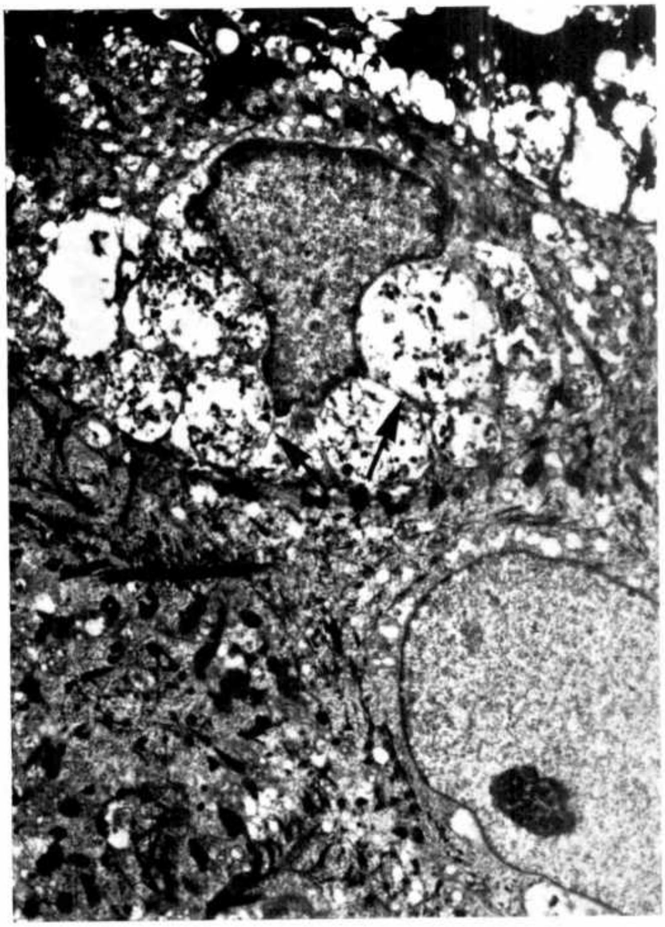

FIGURE 4. A keratinocyte in the granular layer beneath the cornoid lamella. The cell shows typical degenerative features with dense granules, cytoplasmic strands (arrows) between the pyknotic nucleus and the cell membrane, and large vacuoles. Case 2 (× 4900).

underwent this degenerative change retained the rounded shape characteristic of cells in the lower layers of the epidermis, even when they had moved into the stratum corneum (Fig. 5).

Within the cornoid lamella itself, many of the cells still contained complete but pyknotic nuclei and pockets of cytoplasmic debris within vacuoles (Figs. 5 and 6). Large amounts of keratin-like material were present, but this did not exhibit the typical features of either keratohyalin or of mature fibrous keratin. The intercellular material was similar to that seen in normal stratum corneum and was present between both rounded and flattened cells (Fig. 6).

Some areas of the dermis showed more extensive abnormalities than could have been predicted from the light microscope findings. These abnormalities were patchy, however; some parts were completely normal, and the abnormal areas in the dermis were not confined to the region beneath the cornoid lamella.

Many fibroblasts showed degenerative changes and contained large vacuoles, distended endoplasmic reticulum and unusually dense nuclei (Fig. 7). Both fibroblasts and macrophages contained

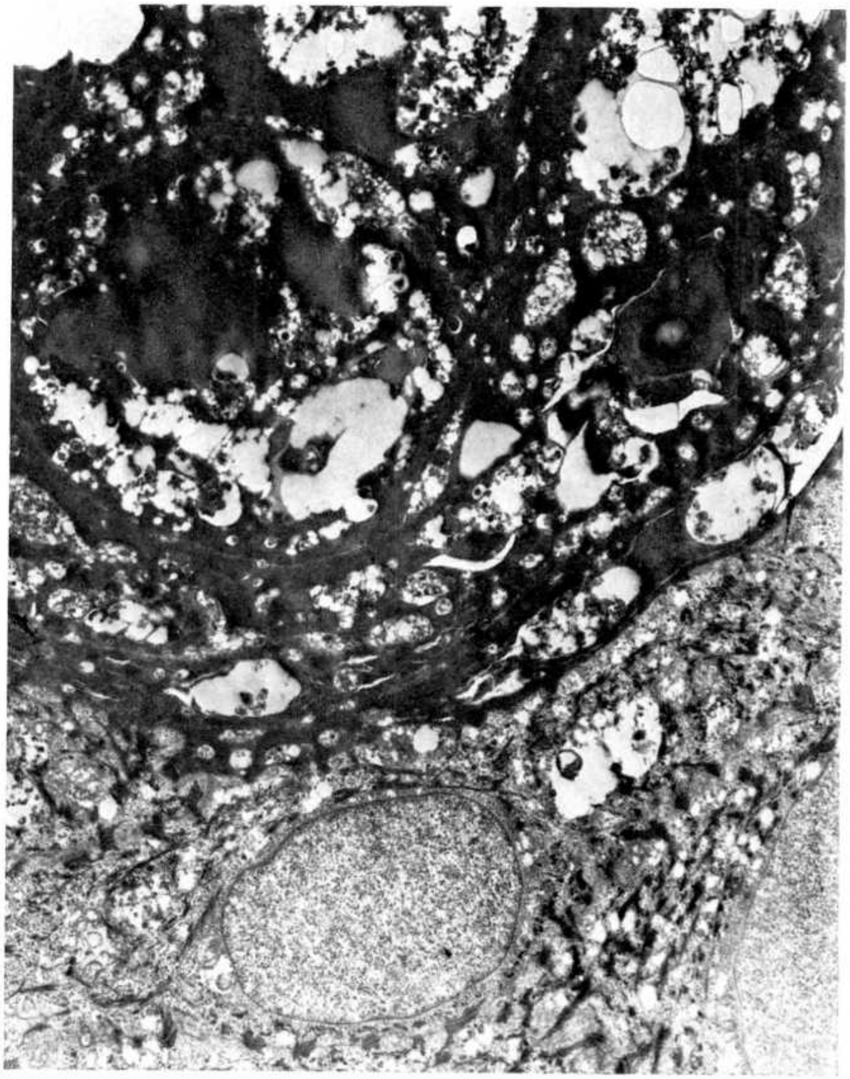

FIGURE 5. Part of a cornoid lamella containing rounded and flattened cells, both of which contain large amounts of cytoplasmic debris in vacuoles. Case 1 (× 4500).

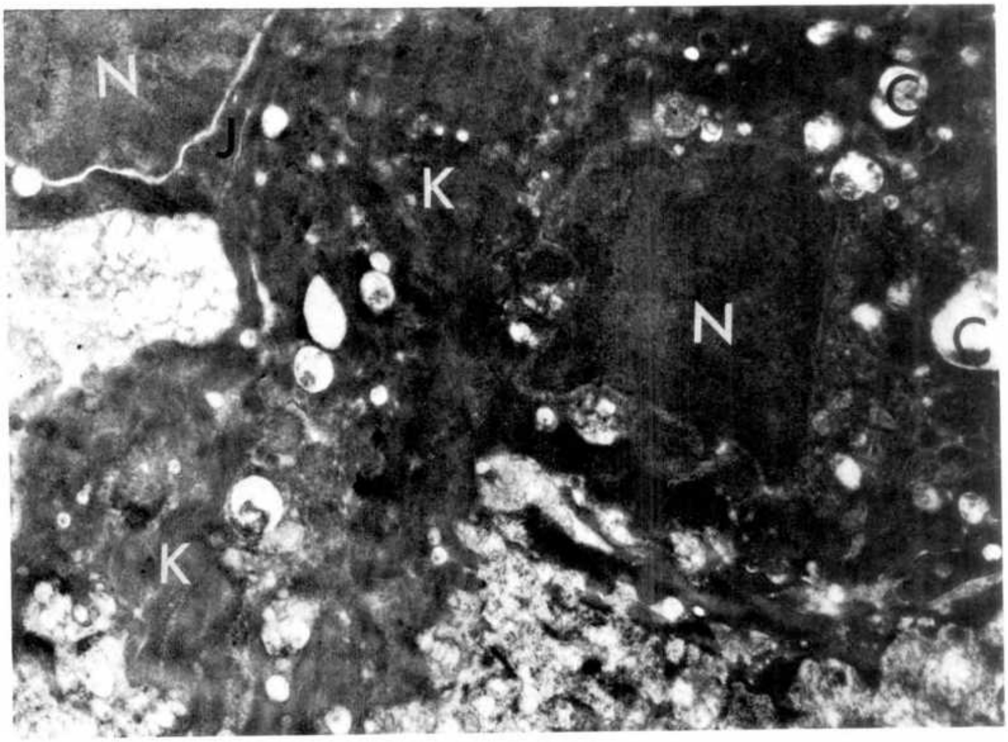

FIGURE 6. Rounded cells with intact, pyknotic nuclei (N) in the cornoid lamella. Cytoplasmic debris (C) is also present, as are dense keratin (K) and normal cell junctions (J). Case 2 (× 11,000).

phagocytosed melanin granules (Fig. 8) and degenerate collagen (Fig. 9). Other cellular components of the dermis were apparently normal in structure and distribution.

Much of the collagen appeared to be normal, but some bundles contained fibrils of variable size and there were foci of degeneration (Fig. 10). Quite frequently, large masses of material with a fine fibrillar structure were seen. The largest of these fibrils was of the order of 15 $m\mu$ in diameter. Collagen fibres, singly and in small bundles, were interspersed with this substance, as were occasional fibroblast processes (Figs. 7 and 8).

DISCUSSION

Mibelli's original hypothesis (1893) that the fundamental lesion in this porokeratosis is hyperkeratosis of the sweat pore and the associated epidermal portion of the duct no longer holds credence. Histological and histochemical studies have not supported the theory, but its actual pathogenesis remains obscure. In a recent exhaustive histochemical study, Braun-Falco & Balsa (1969) showed conclusively that the cornoid lamella was not necessarily derived from the sweat pores, and considered the term suggested by Miescher (1941) of 'parakeratosis centrifuga atrophicans' was more appropriate than porokeratosis. They gave an exhaustive table of the histochemical reactions of various parts of the cornoid lamella, concluding that all the staining techniques indicated that this was a true parakeratosis and that the basophilic material was of nuclear origin. This conclusion is supported by our findings of whole degenerate cells with pyknotic nuclei and dying cytoplasm in the cornoid lamella. The amounts

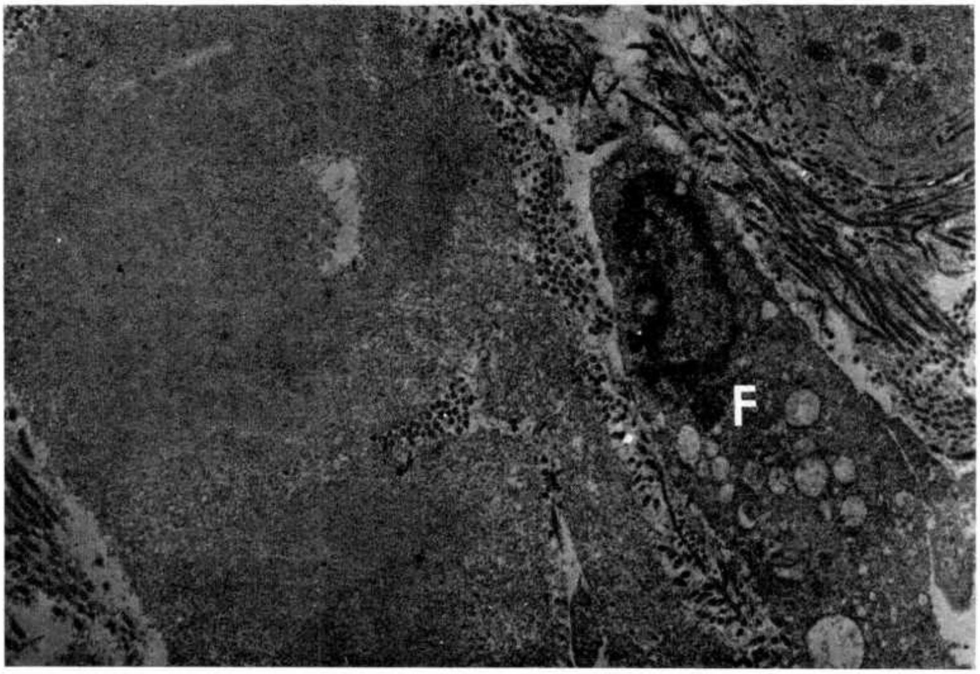

FIGURE 7. An abnormal area in the dermis with a vacuolated fibroblast (F) and a large mass of finely fibrillar material. Case 1 (× 6000).

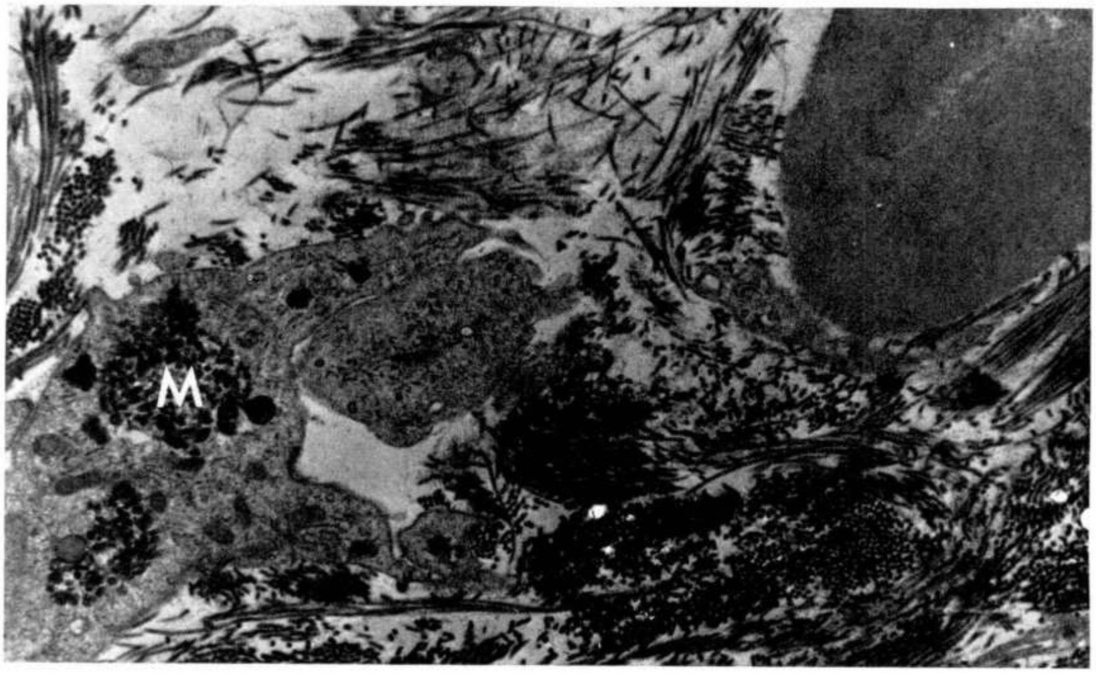

FIGURE 8. A dermal macrophage containing phagocytosed melanin (M) adjacent to another fibrillar mass. Case 2 (× 6200).

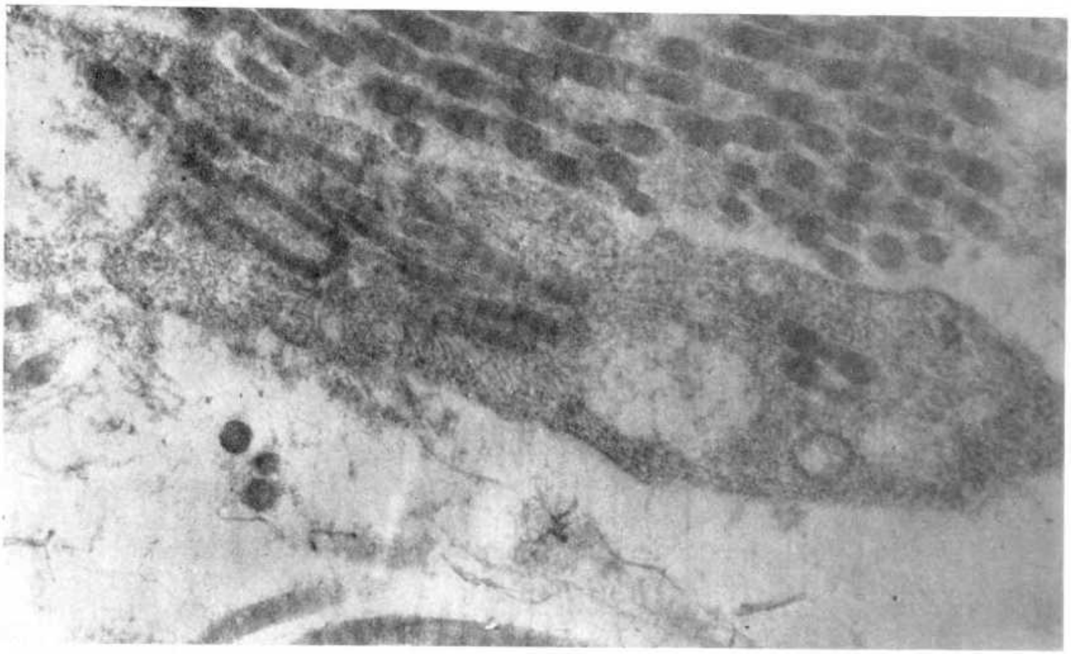

FIGURE 9. A fibroblast process containing phagocytosed collagen. Case 1 (× 43,600).

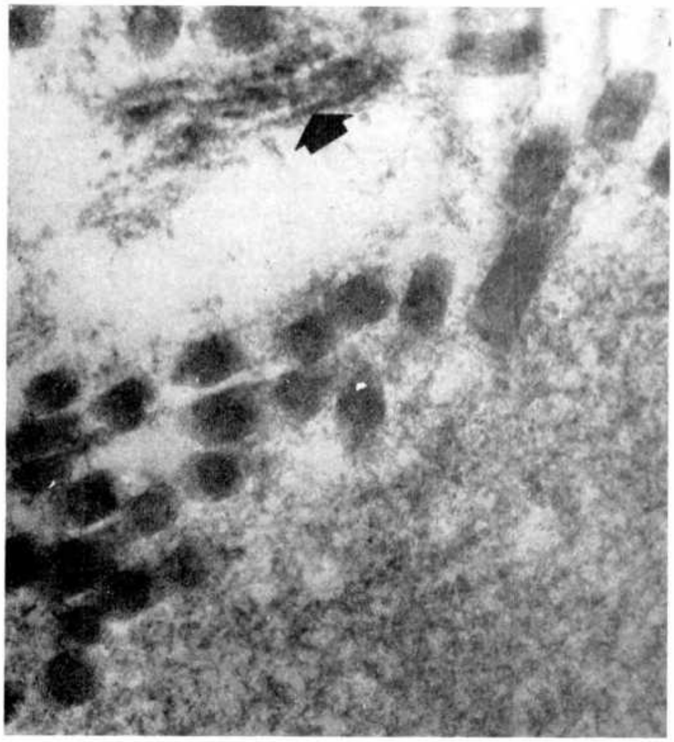

FIGURE 10. Degenerating collagen (arrow) and apparently normal collagen fibrils very close to a fibrillar mass. Case 1 (× 60,000).

of cytoplasmic and nuclear debris and the extent of vacuolation present in cells in the stratum corneum are much greater than are found in other skin conditions involving abnormalities of keratinization in which vacuolation of the stratum corneum is found, e.g. psoriasis (Brody, 1962, 1963; Hanusova, and 1961), pityriasis rubra pilaris (Cruickshank & Mann, 1971). The lack of normal amounts of keratohyalin probably contributes to the failure of cells to keratinize normally, although the lack of a granular layer in psoriasis does not result in an abnormality similar to that found in porokeratosis of Mibelli.

Considering the extent of the abnormalities in the cornoid lamella, it is surprising that the rest of the epidermis was almost entirely normal. The presence of large numbers of melanin granules in dermal fibroblasts and macrophages does, however, indicate that there has been breakdown of the basal lamina in some areas and damage to some melanocytes. The extensive replication of the basal lamina in some areas is similar to that seen in several skin diseases, e.g. psoriasis (Cox, 1969), pityriasis rubra pilaris (Cruickshank & Mann, 1971), following application of hexadecane to produce epidermal hyperplasia (Cowan & Mann 1971), and in the early stages of carcinoma developing in methylcholanthrene treated mice (Tarin, 1967). This response of the basal lamina in various conditions is probably nonspecific. However, the basal lamina may influence the undoubted interaction between dermis and epidermis and in view of the dermal abnormalities and the precancerous potential of the disease, abnormalities at the dermal–epidermal junction cannot be entirely dismissed as insignificant.

The fine fibrillar dermal deposit could not be positively identified, although those fibrils which could be measured were within the limits given for amyloid by Brownstein & Hashimoto (1972). There was not the association of this material with fibroblasts as described by these authors, nor were the bigger fibres they describe seen. There were, however, definite foci of collagen breakdown which were sometimes very close to the large fibrous masses. In addition, phagocytosed collagen was present in some fibroblast processes, which indicates collagen instability. These facts suggest that this finely fibrillar material is derived from degeneration of collagen rather than from the deposition of some newly formed material.

An intrinsic dermal abnormality has been postulated as the primary lesion in the disease (Ritchie & Becker, 1932; Verotti, 1933), but Reed & Leone (1970) stated that the dermal changes are variable and secondary to an epidermal abnormality. Braun-Falco & Balsa (1969) did not commit themselves in relation to the significance of the dermal changes, but pointed out that these occur not only in relation to the cornoid lamella but also in other apparently normal areas, as we have also found.

The degenerative appearance of some fibroblasts may be a reflection of the chromosomal instability found in fibroblasts cultured from these patients (Taylor *et al.*, 1973), and abnormalities in the fibroblasts almost certainly contribute to the disorganization of collagen which was seen.

The influence of dermis upon the development of squamous cell carcinomata has been considered of importance by several workers (Orr & Spencer, 1972; Steinmuller, 1971) and in view of this it is suggested that the role of the dermis in the pathogenesis of the disease could profitably be further investigated.

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We would like to thank Mr J. R. Cooper for taking the light photomicrograph, and Mr A. J. Burton for technical assistance with the electron microscopy.

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A surface microscope for clinical and laboratory use

W.J.CUNLIFFE, R.A.FORSTER AND M.WILLIAMS

The Departments of Dermatology, The General Infirmary, Leeds,
and Leeds (St James's) University Hospital

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SUMMARY

A technique of surface microscopy has been developed which gives good visualization of the skin, is reasonably inexpensive, is easy to use, and is readily adapted for micrometry and photography.

Dermatologists can still learn a great deal by a detailed *in vivo* examination of the surface of the skin. To this end, clinicians have tried to devise a method of examining the skin, which would be an improvement on the naked eye or hand lens examination, and yet would not require a biopsy.

Skin surface biopsy using a cyanoacrylate gel (Marks & Dawber, 1971), and skin replication using Silflo dental plastic (Sarkany, 1962) are just two of the methods developed. We sought to develop a technique which gave good visualization of the skin surface, and which was convenient for use in an out-patient department.

There were three basic problems—the choice of microscope, the type of lighting and the preparation of the skin.

Initially we used a monocular microscope which was adapted to fit an angle-poise stand; this, however, was cumbersome, unsteady and distinctly uncomfortable for the operator to use for any length of time. The major disadvantage, however, was the difficulty in focusing the microscope on any area of the back or chest, due to respiratory movements. We tried many other microscopes, all of which proved unsuitable either because of limitations of depth of focus, inadequate lighting or excessive size or weight. With some instruments, even though the image was good, lack of adaptability allowed only limited sites to be examined. Furthermore, the patient suffered discomfort from being obliged to remain relatively still for long periods of time.

Eventually we investigated the Leitz MZ microscope.* This is normally used for examining the surface of metals, and magnifies either 25 times or 50 times (Fig. 1). This instrument is light and compact, and has two major advantages. It is simple to keep the site in focus, the microscope being hand held on the skin, and therefore moving in time with the patient's respiratory movements. Secondly, it is so adaptable that it can be used on any skin site (Fig. 2).

With regard to the choice of illumination, we found that too bright a light resulted in excessive glare on the skin and no advantage was obtained by using a polarizing filter. A light attachment is available with the Leitz MZ microscope. This gave sufficient illumination, but it reduced the adaptability of the instrument, and so we found that general illumination with a fluorescent lamp, as provided by One Thousand and One Lamps Ltd, proved to be the most suitable.

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* E. Leitz (Instruments) Ltd, 48 Park Street, Luton LU1 3HP.

Although the Leitz MZ microscope is not ideal for normal skin, it gives good visualization in many common dermatoses, such as psoriasis, eczema, ichthyosis, warts, purpura and haemangiomas (Plates 1–4). Perhaps it goes without saying that in Leeds we are also interested in the pilo-sebaceous areas. Clearly, therefore, we have investigated the possible application of this method in these sites. We have found that to obtain the best image in these areas the skin has to be prepared by stripping twice with 3M Sellotape and then stained with a lipophilic stain (saturated solution of Oil Red O in 70% alcohol/acetone). This technique removes the surface glare, which otherwise is a problem.

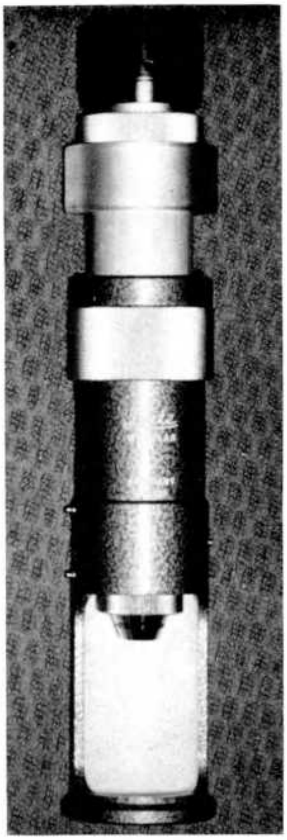
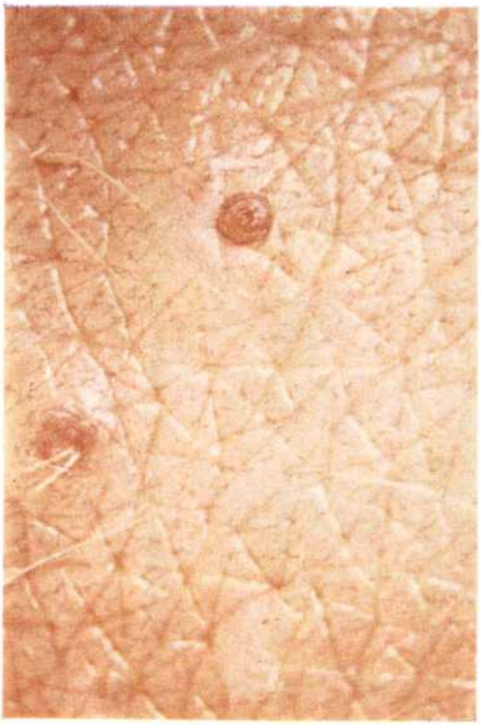


FIGURE 1. The Leitz MZ surface microscope.

A graticule can be incorporated into the eye-piece of the microscope, allowing measurements of scales, pilo-sebaceous duct orifices, and so forth, to be easily obtained. In addition, the Leitz microscope can be adapted for photographic purposes enabling photographs to be obtained, even in inexperienced hands. We have adapted a Leica MDa body and Leicaflex view finder to the microscope (Fig. 3), and by using high speed Ektachrome film ASA 160 and a multiblitz 50E flash gun at a distance of 2 feet, have obtained acceptable results.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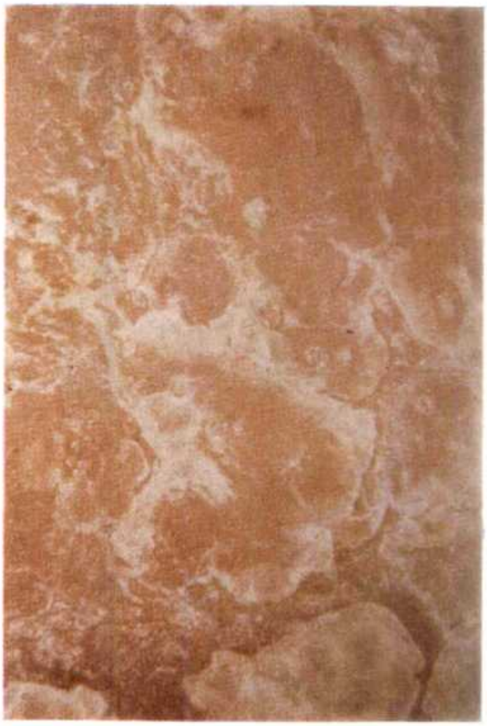
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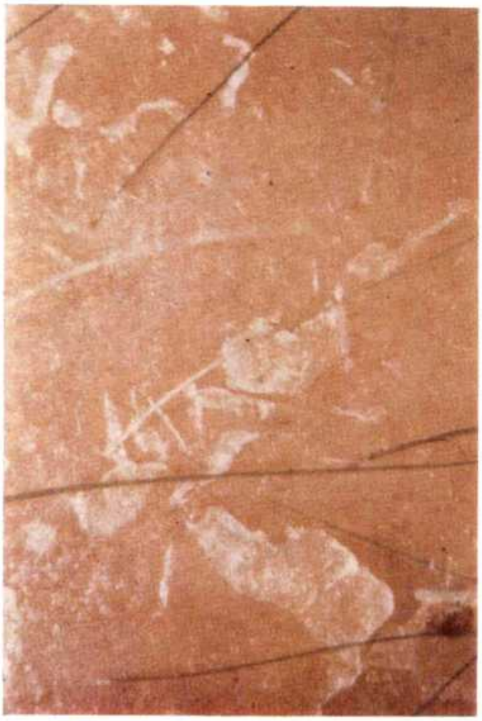
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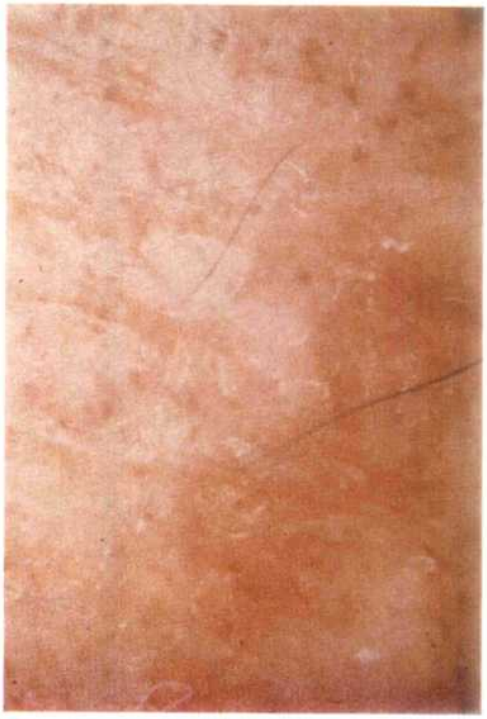
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PLATE 1. Purpura (× 20).
PLATE 2. Psoriasis (× 20).
PLATE 3. Eczema (× 20).
PLATE 4. Area of back showing pilo-sebaceous duct orifices and comedones (× 20).

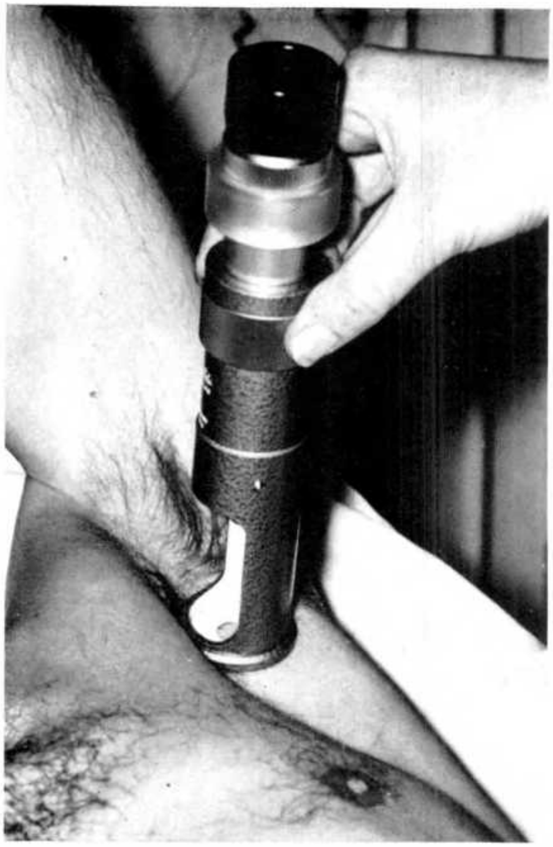

FIGURE 2. The microscope in use.

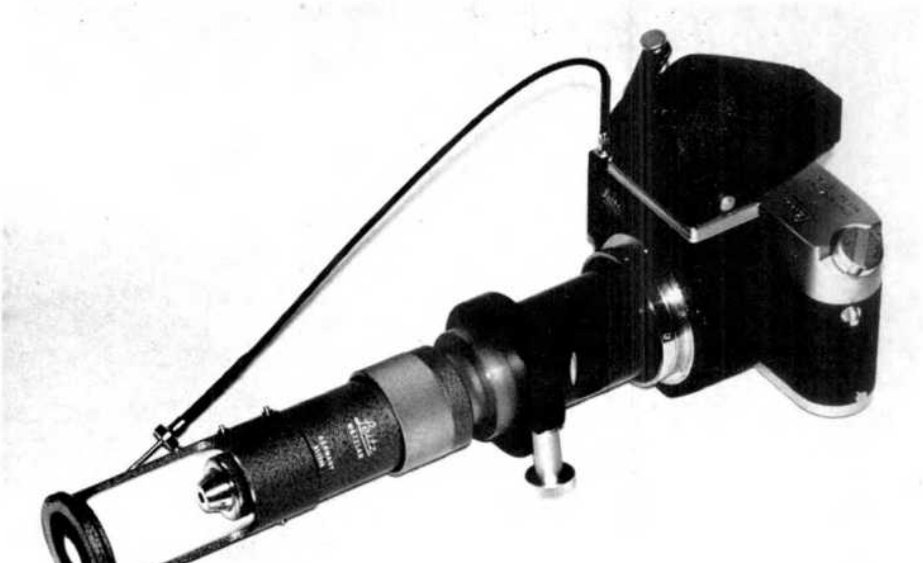

FIGURE 3. Microscope adapted for photographic purposes.

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Acne vulgaris: proliferative cells in sebaceous glands

G. PLEWIG

Department of Dermatology, University of Munich, 8 München 2, Frauenlobstrasse 9, Germany

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SUMMARY

The proliferative activity of sebaceous glands of uninvolved skin from acne patients was analysed by means of ^{3}H-thymidine autoradiography and by planimetry. Twelve specimens of normal skin from the back (with thirty-five glands) and two specimens from the face (with six glands) were analysed. Labelling indices and planimetric values were determined separately for the two portions within each gland: the differentiating cell pool with germinative cells and large lipid cells; and the undifferentiated cell pool with multilayered cells.

The germinative cells of the differentiating cell pool on the back and face of patients with acne showed a significantly greater labelling index than in control subjects. The undifferentiated cell pool, although having a higher labelling index than the differentiating cell pool, was similar both in acne patients and in controls. Planimetry confirmed that the sebaceous glands of patients are much larger than those of normal subjects. Therefore, the known increase in sebum excretion in acne patients is a result of two factors—an increase in lipid cell production and an increase in sebaceous gland size.

More and more evidence is available showing that patients with acne vulgaris have a higher sebum excretion than matched controls (Cotterill, Cunliffe & Williamson, 1971; Cunliffe *et al.*, 1972; Cunliffe & Shuster, 1969a, b; Gloor *et al.*, 1972; Pochi & Strauss, 1954; Powell & Beveridge, 1970; Strauss & Pochi, 1961). Measurements have been based upon collection of surface lipids and on gravimetric assays. They merely reflect the pooled excretion of all sebaceous glands within the collection site.

Increased sebum excretion can be attributed to (1) an increased proliferation of lipid cells, (2) larger sebaceous glands, (3) more sebaceous gland bearing follicles in acne-prone skin sites, or (4), any combination of these possibilities.

To study the first possibility, the proliferative activity (Labelling Index, LI) of human sebaceous glands was analysed by means of tritiated-thymidine (^{3}H-TdR) autoradiography. The results were compared to those control values in normal male subjects which had been reported previously (Plewig, Christophers & Braun-Falco, 1971a).

MATERIALS AND METHODS

Uninvolved skin from forehead ($n = 2$) and upper mid-back ($n = 12$) of male acne vulgaris patients (age 18–24) with a grade I–III acne (Pillsbury, Shelley & Kligman, 1962) was selected. Areas with acne lesions (comedones, papules, pustules) were omitted to avoid including inflammatory changes which greatly influence cell proliferation.

A suspension of 5 μCi of ^{3}H-TdR (thymidine methyl H3, The Radiochemical Centre, Amersham) in 0·1 ml of physiological saline, was injected intradermally between 9 and 10 a.m. 45 min later excisional biopsies were taken under local anaesthesia. Specimens were fixed in Bouin's solution, prepared for autoradiography by the dipping method with 19–21 days exposure, and processed as previously described for similar experiments (Plewig *et al.*, 1971a).

Two types of quantitative evaluation of the specimens were performed: cell counting and planimetry. Sebaceous glands of sebaceous follicles but not those of vellus hair follicles were used; adequate labelling both of the overlying epidermis and of the pilosebaceous structures ensured that satisfactory labelling had been achieved.

Evaluation started with planimetry of semi-serial sections of individual glands, projected against a wall with a Leitz-Prado slide projector and a Leitz micrometer slide table at a constant magnification of 400:1, according to a previously described technique (Plewig *et al.*, 1971a).

After planimetry, the same areas within the specimens were identified under a light microscope, and cell counts were performed under 400-fold magnification.

Sebaceous gland cells were grouped according to their anatomical position and cellular morphology. Counts were made for:

The differentiating cell pool (DCP)
(1) The number of germinative cells.
(2) The number of ^{3}H-TdR-labelled cells.

Aside from this, a portion of the gland consisting of groups of undifferentiated multilayered cells, called the undifferentiated cell pool (Plewig *et al.*, 1971a, b), was evaluated separately. Counts were made for:

The undifferentiated cell pool (UCP)
(3) The number of basal cells.
(4) The number of suprabasal cells.
(5) The number of ^{3}H-TdR-labelled cells in this compartment.

The planimetric evaluation of the sebaceous gland consisted of two separate measurements according to the above-mentioned groupings:
(6) The area of the germinative and lipid cells (DCP).
(7) The area of the undifferentiated cell pool.

The labelling index was determined separately for the DCP and the UCP.

For all identifiable sebaceous follicles in a specimen (ranging from 1 to 5 per section), the data were tabulated separately to evaluate differences in gland proliferation within one site.

The data were statistically examined by Student's *t*-test and the Man–Whitney U-test. The second test was used as a check on the *t*-test, because there was doubt that the underlying assumptions of the *t*-test were satisfied by all of the data. Since the two tests were in close agreement in each comparison, the results of the *t*-tests have been presented. The correlation coefficient (*r*) and the slope of the line fitted by least squares were also calculated where appropriate (Snedecor, 1962).

RESULTS

Cell counts, LI and planimetrical areas are shown in Table 1.

TABLE 1. Cell counts, labelling indices in % and planimetric areas in cm² for the differentiating cell pool and the undifferentiated cell pool of back and face

	Differentiating cell pool (DCP)				Undifferentiated cell pool (UCP)				
Specimen	Germinative cells	Labelled germ. cells	LI(%)	Area (cm²)	Basal cells	Suprabasal cells	Labelled basal cells	LI(%)	Area (cm²)
Back									
1a	1013	121	11·9	1990	44	72	12	27·3	12
b	996	112	11·2	1337	*	*	*	*	*
c	439	62	14·1	627	*	*	*	*	*
d	1082	157	14·5	1578	*	*	*	*	*
			12·9 ± 1·6						
2	1182	218	18·4	1906	20	21	13	65·0	47
3a	1668	220	13·0	3900	90	238	24	26·7	232
b	1362	162	11·9	2231	206	320	49	23·8	126
			12·5 ± 0·8					25·3 ± 2·1	
4	1480	242	16·4	3712	*	*	*	*	*
5a	935	86	9·2	1322	179	281	45	25·1	85
b	925	87	9·4	1158	79	105	17	21·5	34
c	1069	73	6·8	1245	334	822	48	14·4	239
d	1001	83	8·3	1101	82	205	15	18·3	75
e	942	111	11·8	1254	76	116	19	25·0	56
			9·1 ± 1·8					20·9 ± 4·6	
6a	1498	147	9·8	3595	152	219	38	25·0	47
b	1482	127	8·6	2908	86	109	19	22·1	40
			9·2 ± 0·8					23·6 ± 2·1	
7a	1075	61	5·7	1589	156	221	33	21·2	65
b	1396	134	9·6	2214	48	101	25	52·1	26
c	935	76	8·1	2042	107	175	56	52·3	97
d	1724	192	11·1	3376	87	120	13	14·9	31
			8·6 ± 2·3					35·1 ± 19·9	
8a	939	76	8·1	896	72	117	10	13·9	29
b	1652	191	11·6	3603	472	672	54	11·4	212
			9·9 ± 2·5					12·7 ± 1·8	
9a	1834	200	10·9	1569	*	*	*	*	*
b	751	76	10·1	1156	159	236	20	12·6	79
c	600	74	12·3	930	210	385	32	15·2	135
			11·1 ± 1·1					13·9 ± 1·8	
10a	1342	108	8·0	2041	201	346	33	16·4	131
b	664	56	8·4	915	41	45	3	7·3	15
c	783	52	6·6	1540	269	317	22	8·2	77
			7·7 ± 0·9					10·6 ± 5·0	
11a	547	48	8·8	930	193	238	20	10·4	114
b	208	31	14·9	462	88	170	16	18·2	84
c	130	15	11·5	376	71	141	20	28·2	69
d	302	28	9·3	565	404	614	38	9·4	190
			11·1 ± 2·8					16·6 ± 8·7	

Table 1 (*cont.*)

	Differentiating cell pool (DCP)				Undifferentiated cell pool (UCP)				
Specimen	Germinative cells	Labelled germ. cells	LI(%)	Area (cm²)	Basal cells	Suprabasal cells	Labelled basal cells	LI(%)	Area (cm²)
12a	1276	186	14·6	1791	102	102	22	21·6	19
b	1037	132	12·7	1150	194	269	37	19·1	46
c	835	86	10·3	610	16	21	2	12·5	4
d	2479	257	10·4	3816	199	250	23	11·6	103
			12·0±2·1					16·2±4·9	
Sum	37583	4087			4437	7048	778		
Mean ± SD			11·5±3·2					23·5±15·4	
Face									
1a	725	75	10·3	1220	*	*	*	*	*
b	756	139	18·4	1291	*	*	*	*	*
c	1233	152	12·3	2716	36	109	15	41·7	142
			13·7±4·2						
2a	1667	337	20·2	3026	223	447	43	19·3	115
b	952	91	9·6	1104	122	241	11	9·0	56
c	1427	83	5·8	1627	112	232	18	16·1	38
			11·9±7·5					14·8±5·3	
Sum	6760	877			493	1029	87		
Mean ± SD			12·8±1·3					28·3±19·0	

* Not present.

Labelling Index (LI)

Back. For the germinative cells of the DCP from uninvolved skin of acne vulgaris subjects a mean LI of 11·5% ± 3·2 was calculated (Table 2). Using Student's *t*-test this is significantly higher ($P < 0·01$) than the previously calculated LI of 8·9% ± 2·3 for normal controls (Plewig *et al.*, 1971a). The LI for the twelve specimens ranged from 7·7 to 18·4%.

When several individual sebaceous glands were available for analysis within the same biopsy, a fairly wide range of the proliferative activity was found even for glands in close proximity. In patient no. 7 for example, glands were characterized by figures of 5·7, 8·1, 9·6 and 11·1%. However, the diversity of figures was not a consistent pattern (Table 1). Other specimens displayed fairly even results, such as seen in patients nos. 3, 6 and 10. The average of the labelling indices giving the DCP for the thirty-five entries in Table 1 is 11·5 ± 3·2.

Face. For the germinative cells of this area an even higher LI of 13·1% ± 0·6 was found (Table 2). The mean of the acne group is significantly larger than the mean of the normal group (Plewig *et al.*, 1971a) at a level of significance <0·05.

Variations within the glands

Undifferentiated cells in a coral-reef-like formation occurred in all but one specimen (no. 4). As shown previously (Plewig *et al.*, 1971a, b), they have high proliferative activity. In this instance, there were 23·5% labelled cells for the back and 28·8% for the face (Table 3). However, there were no significant differences in acne patients as compared with control subjects (Table 3).

TABLE 2. Labelling Index (LI) in % of the differentiating cell pool (DCP)

	Back		Face	
	Normal* ($n = 26$)	Acne ($n = 12$)	Normal* ($n = 26$)	Acne ($n = 2$)
	8·9±2·3	11·5±3·2	10·1±2·0	13·1±0·6
Student's t-test	$t = 2{\cdot}85$		$t = 2{\cdot}08$	
Probability	$P < 0{\cdot}01$		$P < 0{\cdot}05$	

* From Plewig *et al.* (1971a).

TABLE 3. Labelling index (LI) in % of the undifferentiated cell pool (UCP)

	Back		Face	
	Normal* ($n = 26$)	Acne ($n = 11$)	Normal* ($n = 26$)	Acne ($n = 2$)
	19·2±6·9	23·5±15·4	23·7±5·1	28·8±18·3
Student's t-test	$t = 1{\cdot}18$		$t = 1{\cdot}13$	
Probability	$P > 0{\cdot}24$ (NS)		$P > 0{\cdot}26$ (NS)	

* From Plewig *et al.* (1971a). NS, not significant.

LI and regional variations

Although only small numbers of specimens were available, there was a trend for regional variations in proliferative activity as previously described for normal subjects (Plewig *et al.*, 1971a). For the back, a lower LI of 11·5%±3·2 ($n = 12$ specimens with thirty-five glands) was found than for the face with a LI of 13·1%±0·6 ($n = 2$ specimens with six glands). Again, these figures for acne patients are higher than for controls (8·9%±2·3 back, and 10·1%±2·0 forehead) (Plewig *et al.*, 1971a).

Correlation between cell counts and planimetry

A good correlation between the number of cells (either germinative or labelled cells) and the respective cross-sectional areas could be observed. Fig. 1 shows the relation between labelled germinative cells of the DCP and the planimetrical areas of the back when the values for individual glands are used. Plotted on a semilogarithmic scale, a linear function of the two factors is seen. For the DCP, the numbers used were the thirty-five pairs from Table 1. The logarithms of the areas were used in the calculation of r and the slope.

Sebaceous gland size

The cross-sectional surface area of the ten largest individual sebaceous glands from the back are summarized in Table 4. It can be seen that sebaceous glands of this area with an average of 0·4047 mm^2 are larger in acne than in non-acne persons. The difference is statistically significant (Table 4).

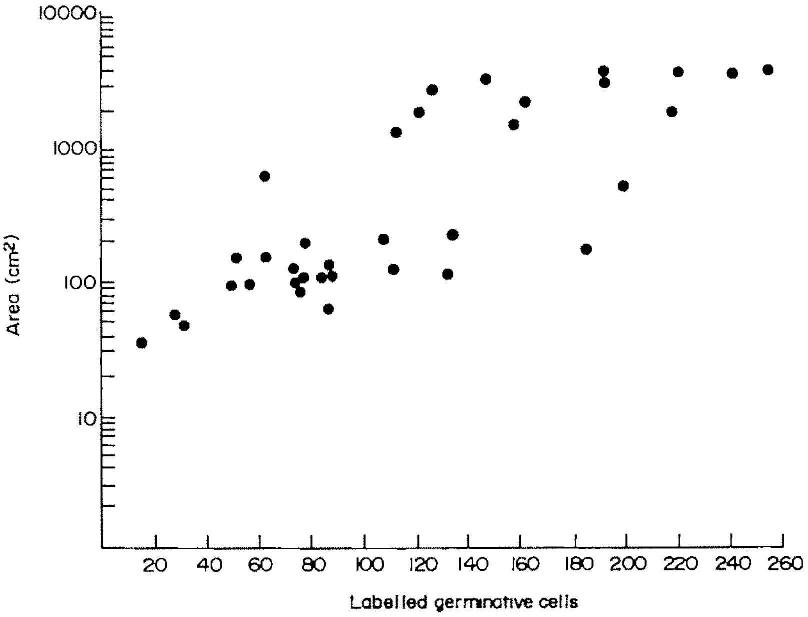

FIGURE 1. Relation between labelled germinative cells (DCP) and planimetry of the respective areas from the back of acne patients. Linear function on a semilogarithmic scale. r = 0·88, slope = 0·001.

TABLE 4. Size of sebaceous glands in mm². The ten largest cross-sectional areas of single sebaceous glands from the back of acne patients were used

	Acne	Normals*
	0·4612	0·2606
	0·2975	0·2993
	0·3412	0·2687
	0·5850	0·2887
	0·3606	0·2981
	0·3693	0·2350
	0·2906	0·2318
	0·4193	0·2306
	0·4750	0·2575
	0·4468	0·3650
Mean ± SD	0·4047 ± 0·0909	0·2735 ± 0·0413
Student's t-test	t = 4·16	
Probability	P<0·01	

* From Plewig *et al.* (1971a).

Size of the undifferentiated cell pool (UCP)

The UCP forms a considerable part of the sebaceous glands. The average share of the UCP of all glands under study from the back is 5·9% (Table 5) compared to 11·7% for normal control subjects (adapted from Plewig *et al.*, 1971a). Since undifferentiated cells are much smaller than the differentiating lipid cells, an even higher numerical share of the former will be expected.

TABLE 5. Percent area of the undifferentiated cell pool (UCP) within sebaceous glands of the back. Measurements from thirty individual glands from twelve biopsies of acne patients (adapted from Table 1) and twenty-six specimens from control subjects

	Normal*	Acne
Mean	11 7	5·9
SD	5·4	5·8

* From Plewig *et al.* (1971a).

DISCUSSION

The pathogenesis of acne vulgaris still remains one of the unsolved problems of dermatology. So far, no anatomical features of the epidermis or pilosebaceous apparatus have been uncovered to explain the development of this disease. However, some differences in functional parameters, such as increased sebum excretion in acne subjects, have been described by several investigators (Cotterill *et al.*, 1971; Cunliffe *et al.*, 1972; Cunliffe & Shuster, 1969a, b; Gloor *et al.*, 1972; Pochi & Strauss, 1954; Powell & Beveridge, 1970; Strauss & Pochi, 1961). The reasons for this augmented lipid production and its role in the aetiopathogenesis of acne vulgaris are unknown. We therefore studied the anatomy of sebaceous glands as well as new gland cell production by ^{3}H-TdR autoradiography.

A higher LI was found for acne patients than for normals (Epstein & Epstein, 1966; Plewig *et al.*, 1971a; Sweeney, Szarnicki & Strauss, 1969). This information was drawn from uninvolved back or facial skin (Table 2). Normal appearing sebaceous follicles with no incipient acne lesions or signs of inflammation were used. The LI for the sebaceous glands of the back was 11·5%, significantly higher than the 8·9% in the controls.

Again, regional variations in proliferative activity of sebaceous glands are evident (Plewig *et al.*, 1971a), the face having a higher LI (Table 2) than the back. Sebaceous glands consist of two different compartments, as previously reported (Plewig *et al.*, 1971a). Undifferentiated cells with no visible lipid droplets at the light microscopical level, form a coral-reef like skeleton throughout the glands. They account for up to 5·9% of the total gland area (Table 4). Their true nature is not exactly known. A high level of proliferative activity, however, seems to be typical for them (Table 3) (Plewig *et al.*, 1971a, b).

The variation we have shown in the proliferative activity of the germinative cells of adjacent sebaceous glands suggests that not all glands function at the same time at the same rate. This behaviour is similar to active or resting hair follicles (Lyne & Short, 1966) or sweat glands (Kuno, 1956; Willis, Harris & Moretz, 1973).

Furthermore, patients with acne vulgaris have indeed larger sebaceous glands than normals (Table 4). Very large sebaceous glands were measured on the back. The mean of the ten largest cross-sectional areas from thirty-five glands was 0·4047 mm^2, which is considerably larger ($P<0·01$) than in normal subjects with 0·2735 mm^2.

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We, therefore, conclude that the increased sebum excretion in patients with acne is due to at least two factors, namely: increased cell proliferation and large sebaceous glands.

ACKNOWLEDGMENTS

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Pilo-sebaceous duct physiology. I

EFFECT OF HYDRATION ON PILO-SEBACEOUS DUCT ORIFICE

M.WILLIAMS, W.J.CUNLIFFE AND D.GOULD

The Departments of Dermatology, The General Infirmary, Leeds,
and Leeds (St James's) University Hospital

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SUMMARY

Using a surface microscope technique to measure the pilo-sebaceous duct orifices *in vivo*, it has been shown that keratin hydration, as produced either by a hot bath or by polythene occlusion, decreases the pilo-sebaceous orifice size. These changes are reversed within 90 min. This reduction in pilo-sebaceous duct orifice size would increase sebum outflow resistance and aggravate duct obstruction. The observations therefore help to explain the exacerbation of acne seen in humid environments, such as in the tropics, and in certain occupations.

Three important groups of factors influencing the development of acne are: (1) sebum excretion rate (Cunliffe & Shuster, 1969), (2) surface lipid composition (Cotterill *et al.*, 1972), (3) sebum outflow resistance (Cunliffe & Shuster, 1969).

Surprisingly, there are few investigations on sebum outflow resistance. It therefore seemed worth while to investigate *in vivo* the effect of physiological variables which hydrate the pilo-sebaceous duct keratin, and thus will probably influence sebum outflow resistance. To this end we used a surface microscope (Cunliffe, Williams & Forster, 1974) which enables us to measure the pilo-sebaceous duct orifice *in vivo*.

MATERIALS AND METHODS

Twenty-eight patients were studied, twenty females and eight males, mean age 19·8 years; all had acne but none had received any treatment whatsoever for at least 6 weeks before the investigation. The pilo-sebaceous duct exits were measured at four constant sites on the upper back; the sites were selected using a template which had its fixed points at the lower angle of the scapula and the mid-line (Fig. 1).

After marking the sites, the outermost keratinocytes were removed by stripping the skin twice with pressure sensitive 3M Sellotape. A lipophilic stain, saturated Oil Red O in equal parts 70% alcohol/acetone, was then applied to the skin and, using a Leitz MZ microscope* with a graticule in the eyepiece, the diameters of the pilo-sebaceous duct orifices were measured.

In all patients the four standard areas were measured immediately before the experimental procedure.

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* E. Leitz (Instruments) Ltd, 48 Park Street, Luton LU1 3HP.

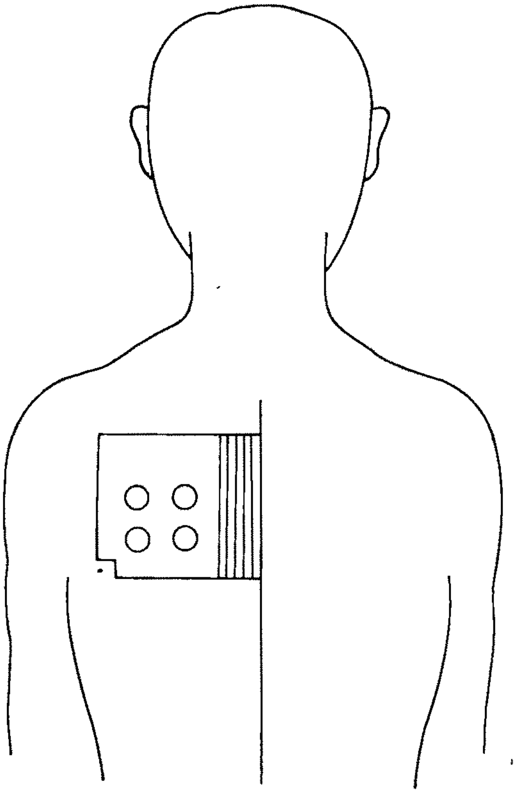

FIGURE 1. Shows the template in position.

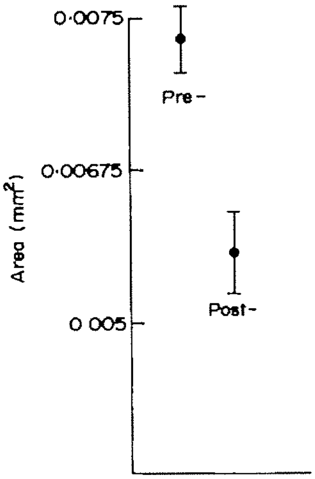

FIGURE 2. The immediate effect of a hot bath on orifice size (mean ± SEM) ($n = 16$, $P < 0{\cdot}025$).

Keratin hydration was produced either by the patient having a hot bath or by applying polythene occlusion to the skin for 24 h. In the hot bath experiment, the patients were immersed up to their loins in water at 45°C for 20 min, but the area under observation was at no time allowed to become wet. The areas were then inspected immediately the patient left the bath; the lower two areas were restained should the original stain have been dissolved as a result of sweating. In eight patients, the same areas were re-inspected at 10–15 min intervals for 90 min.

In a further twelve patients, the four standard areas were prepared and measured immediately prior to their occlusion with polythene for 24 h. Immediately after removing the polythene the areas were again inspected. In five patients, the standard areas were also read at 15 min intervals for 80 min after removing the polythene.

RESULTS

Both the hot bath and polythene occlusion produced a significant reduction in pilo-sebaceous orifice size (Figs. 2 and 3). This decrease reached a trough within 15 min after the patient had left the bath, but the maximum decrease occurred immediately after removing the polythene. In both experiments

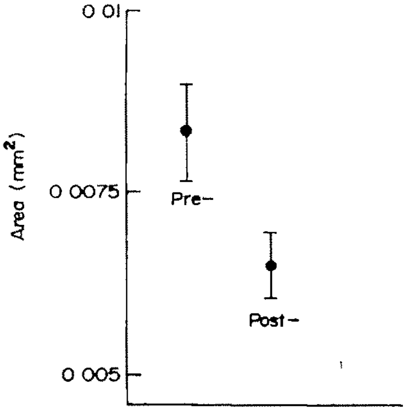

FIGURE 3. The immediate effect of polythene occlusion for 24 h on orifice size (mean ± SEM) ($n = 7$, $P < 0{\cdot}001$).

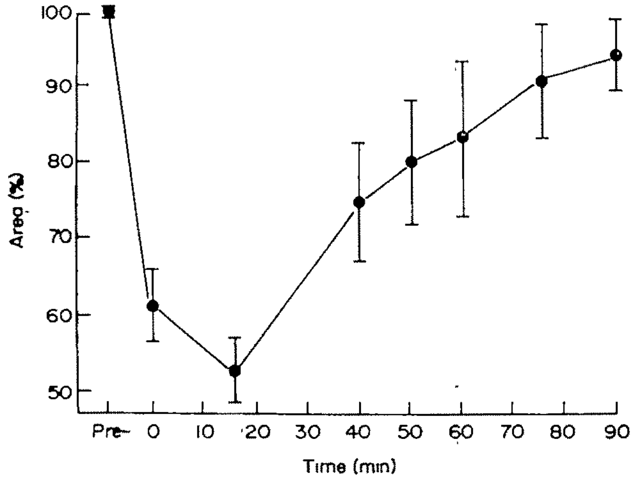

FIGURE 4. Changes in the orifice size (mean ± SEM) in the 80 min after a hot bath ($n = 8$)

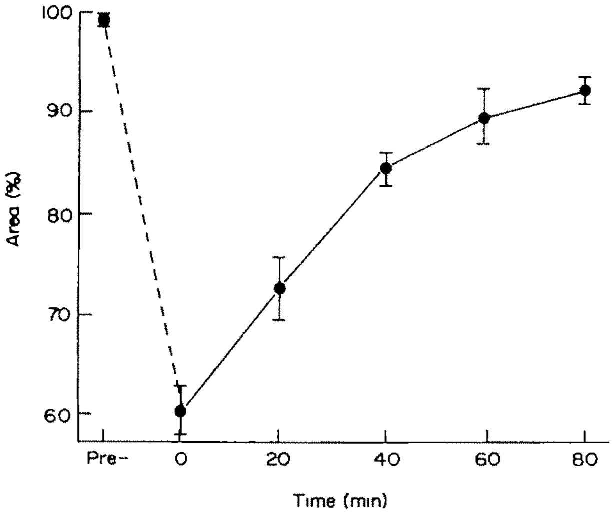

FIGURE 5. Changes in the orifice size (mean ± SEM) in the 90 min after removing the polythene ($n = 5$).

the pilo-sebaceous duct exits returned to their original size 80–90 min after the bath or removal of the polythene (Figs. 4 and 5).

DISCUSSION

The exacerbation of acne seen in the tropics and in subjects working in other humid environments is not adequately explained. Furthermore, it has been observed that 20% of subjects (Cunliffe, 1973) notice a deterioration of their acne in the summer. A common factor linking these clinical situations is increased humidity, which in turn produces keratin hydration. In this present investigation, hydration of the keratin was produced by subjecting the patients either to a hot bath or to polythene occlusion of the skin. In both experiments we demonstrated a significant reduction in pilo-sebaceous duct orifice size, which was reversible after 90 min. The flow of sebum and keratin through the anatomically tortuous pilo-sebaceous duct is a complex problem. We realize that the pilo-sebaceous duct is a complex system, but in simple mechanical terms it does not seem unreasonable to consider our observation in relationship to Poiseuille's formula (Noakes, 1963): $F = (\pi r^4/8\eta) \times (P/L)$ ml/s (F = flow rate, r = radius of tube, η = viscosity, P/L = pressure gradient).

The flow rate of a fluid through a tube is inversely proportional to the outflow resistance, and from Poiseuille's formula is directly proportional to the fourth power of the radius. Clearly, therefore, our observations are of functional importance, for the decrease in pilo-sebaceous duct size with hydration could produce a marked increase in sebum outflow resistance, and precipitate obstruction. Viscosity, which is temperature dependent, is also an important factor in the rate of flow. In both our experiments we produced an increase of skin temperature by 1–3°C. Thus the lowered sebum viscosity could be looked upon as a physiological compensatory mechanism to reduce the sebum outflow resistance, and so to counteract the increased outflow resistance produced by the reduction in the external area of the duct. However, viscosity varies only linearly with temperature and not to the fourth power as does the radius of the system. We admittedly measured only one aspect of the pilo-sebaceous duct, but as this is the exit through which all sebum must pass, changes in its size must inevitably influence sebum outflow. We are now investigating the functional effects of pilo-sebaceous duct occlusion on sebum excretion rate.

We therefore conclude that common physiological variables, such as sweating, can markedly influence the pilo-sebaceous duct size and sebum outflow resistance. These observations help us to understand certain clinical features of acne.

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Ultrastructural studies on tumours and cell cultures of the Harding–Passey mouse melanoma*

STANLEY S.BLEEHEN

Rupert Hallam Department of Dermatology, Hallamshire Hospital and University of Sheffield

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SUMMARY

Ultrastructural studies have been carried out on tumours and monolayers of cells of the transplantable Harding–Passey mouse melanoma, grown in tissue culture. Tumours were found to consist of melanin-producing, melanin-producing-and-containing and melanin-containing cells. Melanosomal complexes found in melanin-containing cells appeared to arise by fusion of solitary melanosomes either with each other or with lysosomes. Melanophages, previously regarded as macrophages which have migrated into the tumour, are probably altered and older melanoma cells. Melanosomal complexes contained in these cells have formed by autophagocytosis. Tissue culture studies support this view. Melanoma cells grown *in vitro* were invariably found to contain melanosomal complexes as well as solitary melanosomes, and older cells in culture contained numerous melanosomal complexes.

Virus-like particles resembling immature C-type virus particles were found in all the tumours examined and were especially abundant in cultured cells. These particles were found in the cisternae of rough endoplasmic reticulum. The significance of these virus particles is still a matter for speculation.

In 1925, Harding & Passey showed that, in a stock of normal mice, one animal spontaneously developed a lobulated mass on the tip of an ear. This they found to be a melanoma. These workers showed that they could transplant this melanoma into several different strains of mice. Though the Harding–Passey melanoma has been extensively studied as a model for biochemical studies on melanin producing cells, morphological information concerning this tumour is limited. In the original report (Harding & Passey, 1930), the tumour was described as consisting of two different types of cells. The predominant cell was large, rounded and heavily laden with pigment which almost obscured the nucleus of the cell. This was regarded as a 'phagocyte', a macrophage which had phagocytosed melanin to become what is now termed a 'melanophage'. Another smaller type of cell which was less pigmented was present. This was found often to be in mitosis and was considered to be the tumour cell proper.

Ultrastructural studies (Novikoff, Albala & Biempica, 1968; Seiji & Otaki, 1971) have shown that the tumour consists of melanin-producing, melanin-producing-and-containing and melanin-containing cells. These studies have suggested that most of the 'melanophages', the melanin-containing cells, are altered and older melanoma cells. The compound melanosomes contained within these cells have resulted from autophagocytosis of melanosomes in a melanin-producing melanoma cell.

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* Presented at the Winter Meeting of the Investigative Group of the British Association of Dermatology, February 1973.

The purpose of this paper is to describe the ultrastructure of the Harding–Passey melanoma; both that of tumours *in vivo* and that of cells grown *in vitro* in tissue culture. Additional information from tissue culture studies provides further support for the view that the 'melanophages' are melanoma cells and not mesenchymal macrophages that have migrated into the tumour.

MATERIALS AND METHODS

The Harding–Passey melanoma was serially transplanted, using the 'chunk' method, into the subcutaneous tissue of the flank of C 57 BL and Swiss strain mice. Small non-necrotic tumours were excised and chopped into fragments and these were immediately fixed in 3% glutaraldehyde. After embedding in Araldite, ultra-thin sections were cut, later stained and viewed in the electron microscope.

Portions of tumour were taken for tissue culture studies. The tumour was mechanically disaggregated to produce a suspension of cells, which after being washed were then seeded into 25 ml falcon flasks. The cells were cultured in 199 medium with 20% foetal bovine serum, to which was added 100 iu penicillin per ml and 100 μg streptomycin per ml. The medium was changed every 5 days. Healthy growing cultures were taken at 1 week and 3 weeks. These monolayers of cells were then fixed with 3% glutaraldehyde and were then embedded in Epon 812 *in situ* in the flasks and ultra-thin sections of selected colonies of cells were cut. After being stained, these sections were viewed in the electron microscope.

RESULTS

Tumours

Light microscopy of tumours showed that they were composed of two types of cells (Fig. 1). One cell was large, rounded and heavily pigmented and contained large pigment granules which often obscured the nucleus. The other cell was less pigmented with a large nucleus often found to be in mitosis (Fig. 1).

Electron microscopy of the tumours showed that apart from necrotic areas the constituent cells had a uniform appearance (Fig. 2). While 'light' and 'dark' cells were seen, this was considered to be a fixation artefact such as has been observed in other tumours. Tumour cells contained solitary melanosomes varying in size from 0·3 μm to 0·6 μm. The melanosomes were rounded in shape and were bounded by a distinct outer limiting membrane. The internal architecture of most of these melanosomes was obscured by the granular electron dense deposits of melanin. Early, less melanized melanosomes, often fusiform in shape, were found and these contained arrays of melanofilaments with a periodicity of particles along their length of 8 nm (Fig. 3). The Golgi apparatus in these cells was well developed and consisted of three to five saccules. Melanosomes in various stages of development were to be found in relation to the Golgi region. A few lamellated bodies, probably representing melanosomes, were found. Ribosomal arrays in spirals and rosettes were present in abundance and the rough endoplasmic reticulum of these cells was prominent.

Frequently found in dilated cisternae of rough endoplasmic reticulum were virus-like particles (VLPs) about 100 nm in size. They appeared to bud off from the ribosomal studded endoplasmic reticulum (Fig. 4). The particles were rounded or semi-circular in shape, with double membranes and usually an electron-lucent core. The appearances of the VLPs were those of immature C-type virus particles. All tumours examined contained these VLPs, which were found only in melanoma cells.

The nuclei of these melanin-producing cells were very large and indented and frequently contained sequestered islands of cytoplasm. Clumping of chromatin was present and large dispersed nucleoli were seen. Other cytoplasmic organelles were found, including structures consistent with and identified as lysosomes, vacuoles, 'myelin' and multi-vesicular bodies.

In addition to melanin-producing cells, melanin-containing cells were found, which contained membrane bounded aggregates of melanosomes—melanosomal complexes (Fig. 5). The melanosomal complexes contained variable numbers of melanosomes which were set in an electron dense granular matrix. The melanosomes contained in these complexes were in various stages of degradation and had lost their individual outer limiting membrane. However, even in melanin-containing cells, individual melanosomes were present. Lysosomal structures were particularly numerous in these melanin-containing cells. They often contained homogeneous electron dense material. Microfocal necrotic changes were observed in the tumour cells with breaks in the continuity of the plasma membrane and extrusion of cytoplasmic organelles into the intercellular space. Degenerate mitochondria, vacuoles and multi-vesicular bodies were seen in these cells.

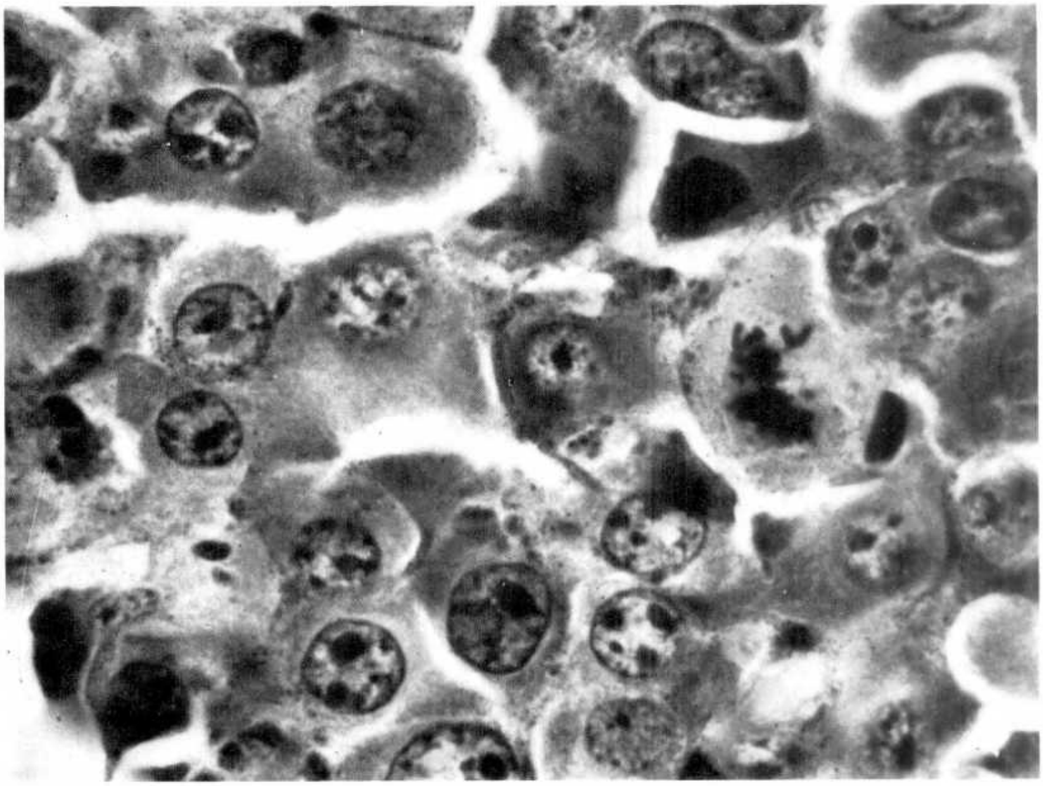

FIGURE 1. Harding–Passey tumour showing melanoma cells (H & E, × 1200).

Tissue culture studies

Monolayers of Harding–Passey melanoma cells grown in tissue culture showed that the majority of the cells were pigmented and dendritic (Fig. 6). Often in older cultures a significant number of more

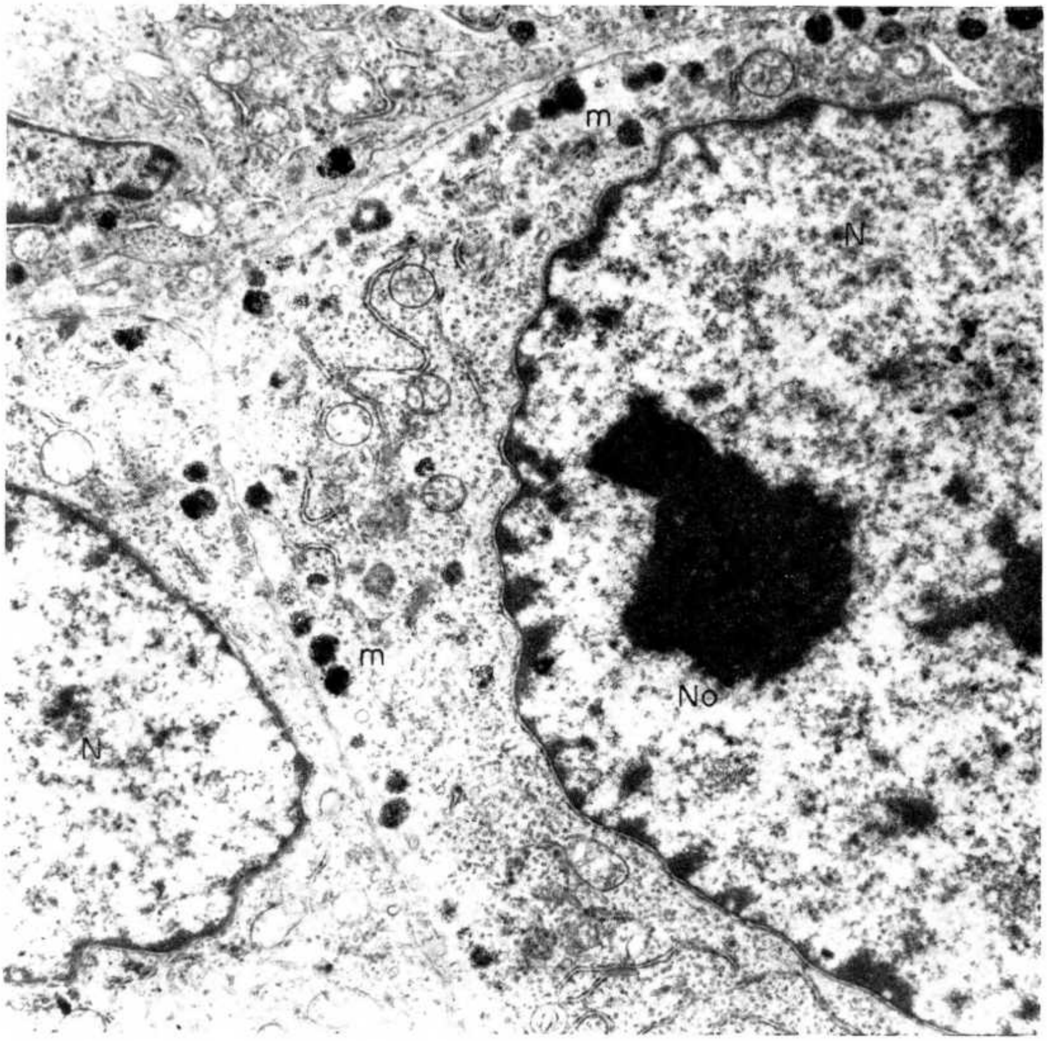

FIGURE 2. Harding–Passey tumour. Electron micrograph showing portions of several melanoma cells. Melanosomes (m), nucleus (N), and dispersed nucleolus (No) (× 7200).

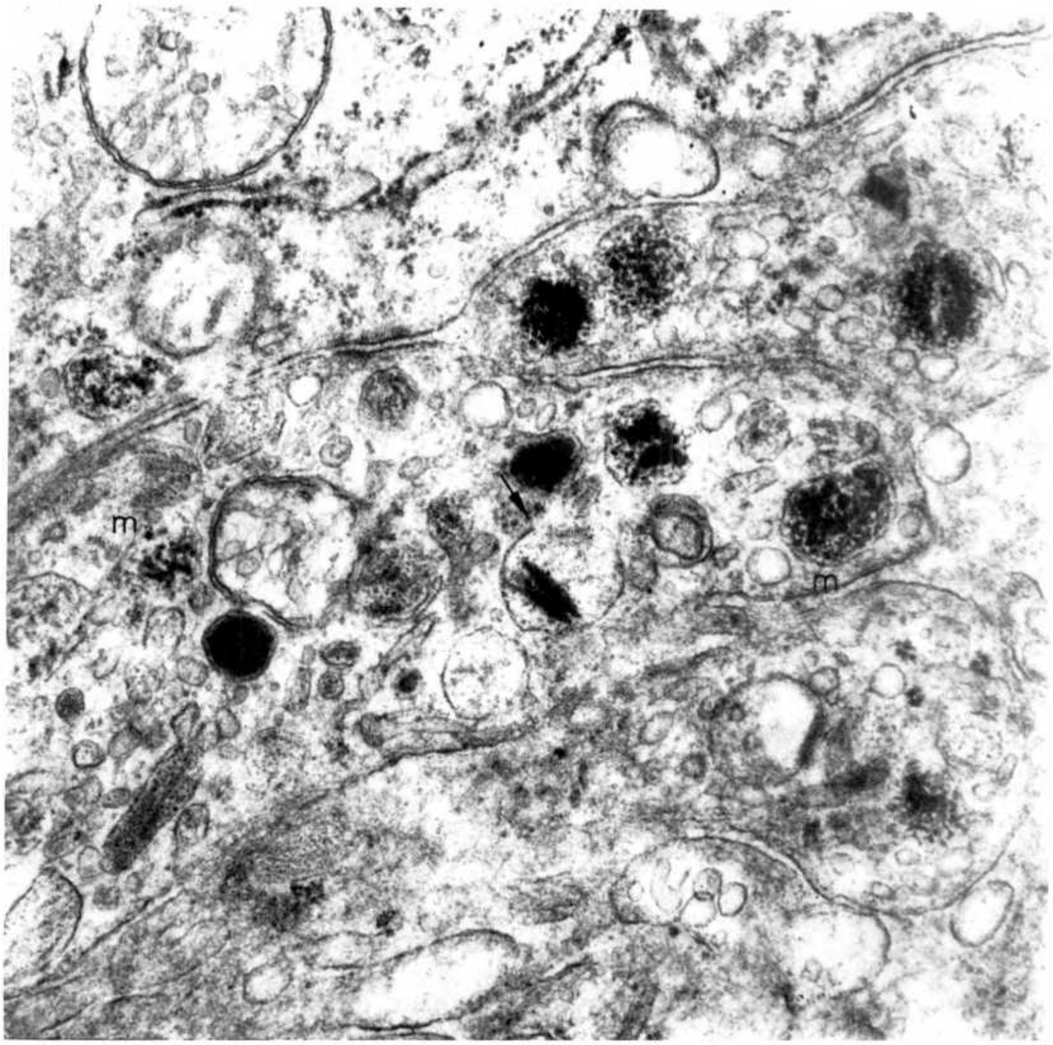

FIGURE 3. Melanosomes (m) in various stages of development. Melanosome (arrowed) in early stage of melanization showing melanofilaments (× 36,000).

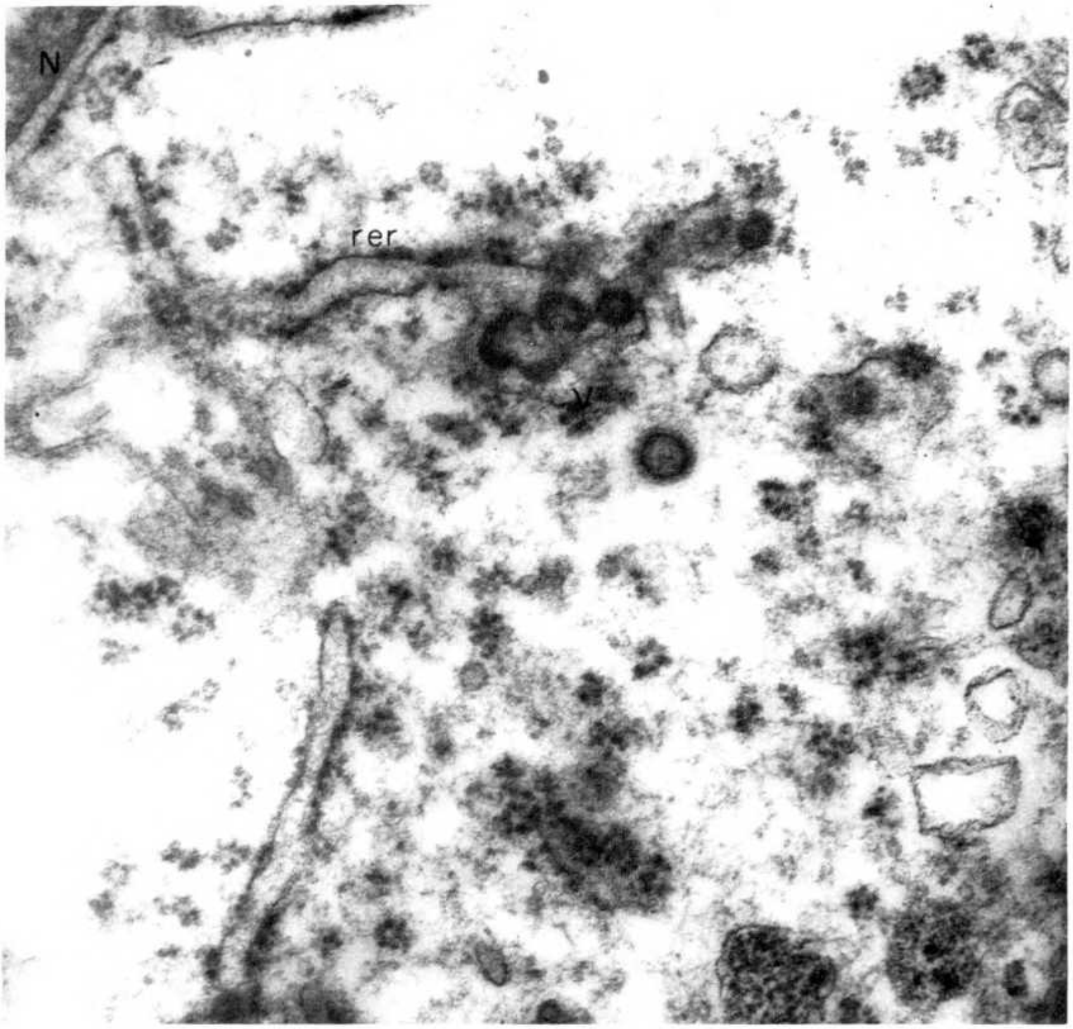

FIGURE 4. C-type virus particles (V) budding off and in cisternae of rough endoplasmic reticulum (rer) and nucleus (N) (× 72,000).

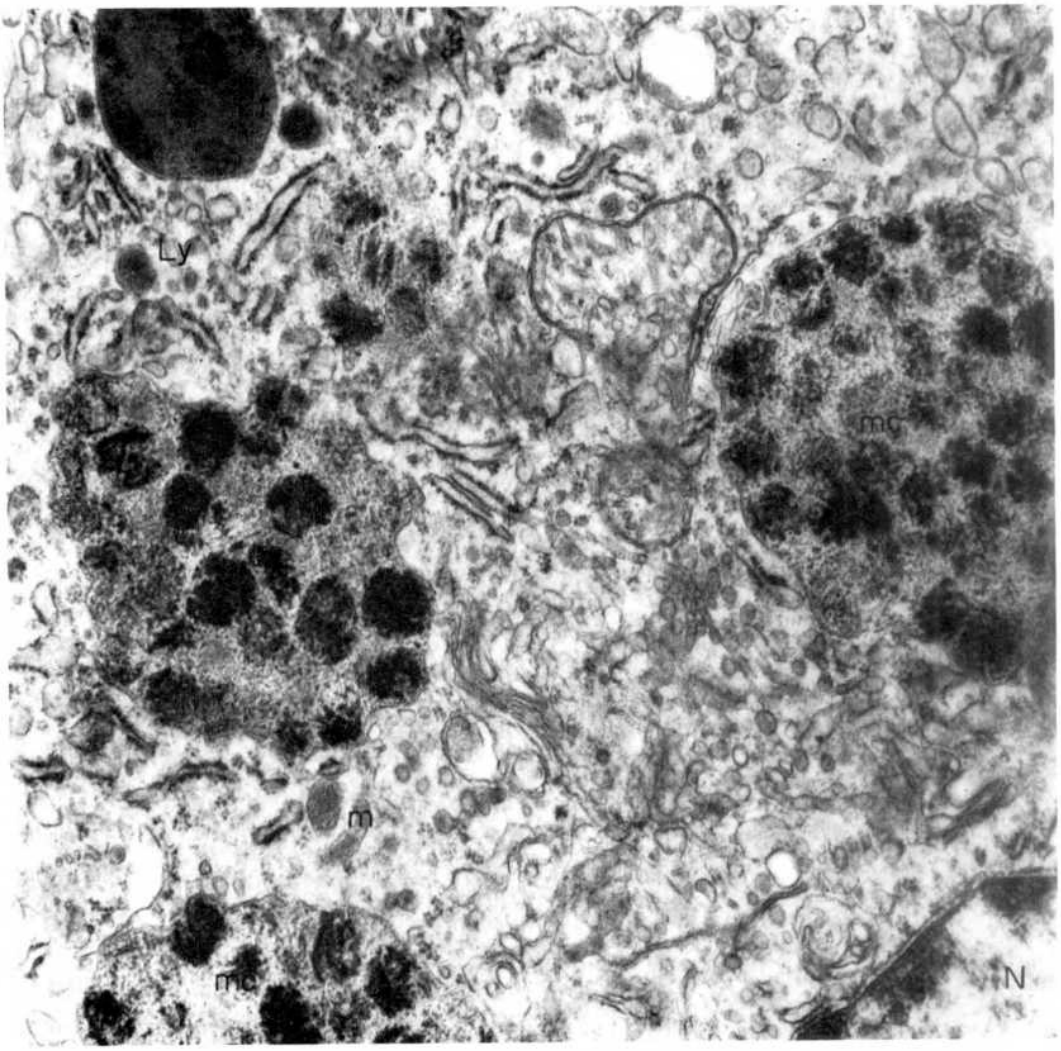

FIGURE 5. Melanin-containing cell with melanosomal complexes (mc) and lysosomal structures (ly). Solitary melanosomes in various stages of development are present (m) (× 28,800).

heavily pigmented, rounded and smaller cells were seen in the central portion of a colony of cells. These cells had lost their dendrites and contained large pigment granules which often obscured the nucleus. Large pigment granules were also found in the more lightly pigmented dendritic melanoma cells.

On electron microscopy, the cells in tissue culture showed a remarkably uniform appearance. The cells often lay in close contact with each other with points of attachment between the plasma membranes of adjacent cells (Fig. 7). The nuclei of these cells were large, lobate with sequestered islands of cytoplasm. The morphology of the melanosomes was as previously described, with solitary melanosomes being bounded by a distinct unit membrane and being arranged towards the periphery of the cell and in dendrites. Lamellated bodies, probably melanosomes, were frequently found (Fig. 7). Melanosomal complexes were invariably found in all the melanoma cells examined. From several to as many as 100 melanosomes were present in these melanosomal complexes. Fusion of the outer limiting membrane of adjacent melanosomes appeared to occur and resulted in the formation of melanosomal complexes. In addition to elongated tubular and branched mitochondria, many microtubules and microfilaments were found in these cultured melanoma cells (Fig. 8).

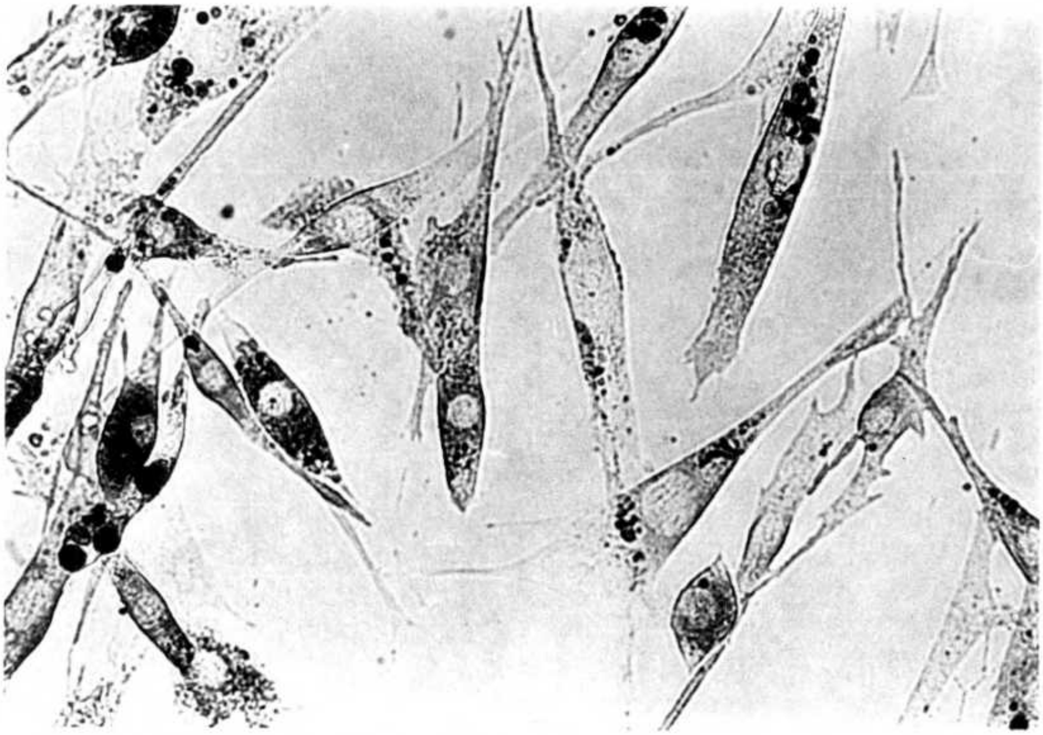

FIGURE 6. Monolayer of cells of Harding–Passey melanoma grown in tissue culture, some containing large pigment granules (× 360).

Many VLPs were present and were invariably found in cisternae of rough endoplasmic reticulum. These VLPs were in abundance, especially in melanoma cells from older cultures (Fig. 8).

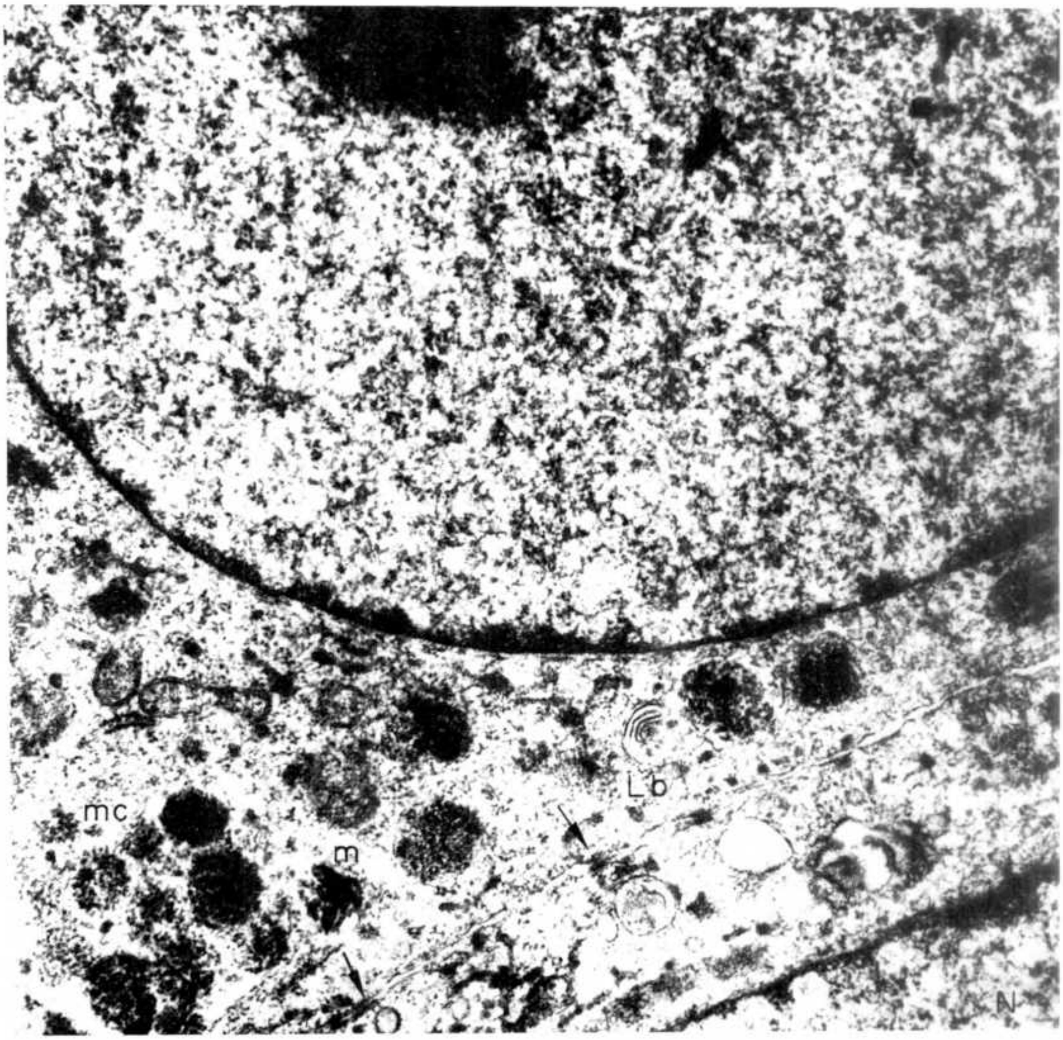

FIGURE 7. Points of attachments (arrowed) between two adjacent melanoma cells grown in tissue culture. Melanosomes (m), melanosomal complexes (mc) and lamellated body (lb) (× 28,800).

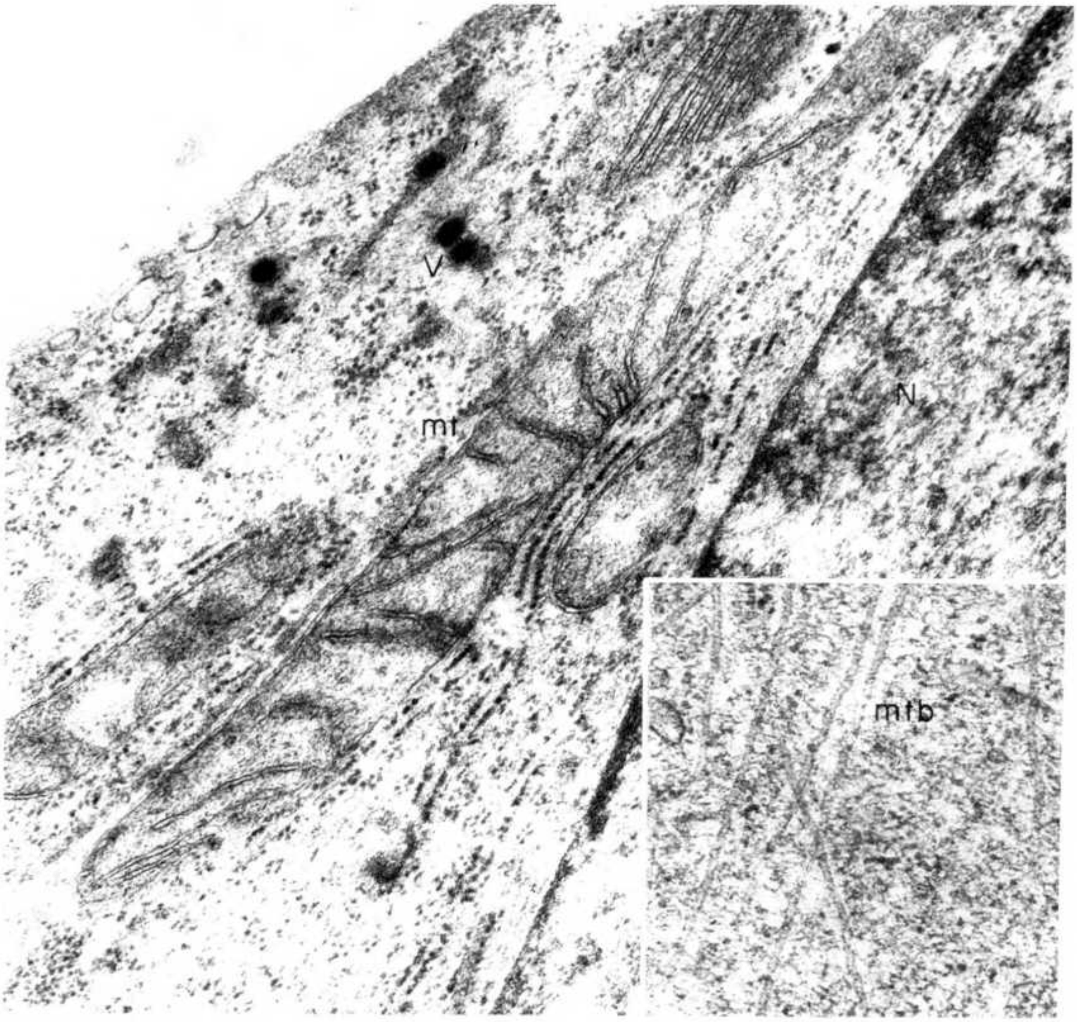

FIGURE 8. Electron micrograph of portions of Harding–Passey melanoma cell in tissue culture showing elongated mitochondria (mt) and virus-like particles (V). N, nucleus (× 36,000). Insert: microtubules (mtb) in cytoplasm of Harding–Passey melanoma cell (× 54,000).

DISCUSSION

These ultrastructural findings confirm previous studies on tumours of the Harding–Passey melanoma (Novikoff *et al.*, 1968; Seiji & Otaki, 1971) and show that they are composed of melanin-producing, melanin-producing-and-containing and melanin-containing cells. Additional support for the view put forward by these workers that the melanin containing cells are altered and older melanoma cells is provided by observations in this study on tissue cultures of Harding–Passey melanoma cells. The cells grown in monolayers in tissue culture were remarkably uniform in their ultrastructure. They contained, in addition to solitary melanosomes, melanosomal complexes, which appeared to arise by autophagocytosis and fusion of melanosomes either with each other or with lysosomal structures. Older cells often to be found in the central portion of large colonies of cells were heavily pigmented, rounded in shape and had lost their dendrites. They contained large and numerous melanosomal complexes but, even in these cells, solitary melanosomes were found.

Recent elegant studies (Wolff & Honigsmann, 1971, 1972), using cytochemical tracers, have indicated that melanosomes can fuse with each other or with lysosomes to form melanosomal complexes. Melanosomal complexes are to be found not only in the cells of the Harding–Passey melanoma, but also in the B16 mouse melanoma (Hu, Swedo & Watson, 1967; Novikoff *et al.*, 1968). The fusion of melanosomes with each other to form melanosomal complexes occurs within these malignant melanocytes. Also, a striking autophagocytosis of melanosomes with the formation of large melanosomal complexes occurs in the surround and nodule cells of lentigo-maligna melanoma (Clark, Ten Heggeler & Bretton, 1972; Bleehen, unpublished observations). The formation of melanosomal complexes in these cells and other melanomas is part of a wide spectrum of subcellular changes in the intracytoplasmic autodegradation of melanoma cells (Mishima & Ito, 1969).

It is most likely that most of the melanin-containing cells, the 'melanophages', seen in the Harding–Passey melanoma, are altered melanoma cells and not mesenchymal macrophages or 'phagocytes' that migrated into the tumour as was suggested by Harding & Passey.

The virus-like particles seen in the cells of tumours and in tissue culture of Harding–Passey melanoma have the appearance of immature C-type virus particles (Bernhard & Granboulan, 1962). These VLPs have been previously noted in the Harding–Passey melanoma (Novikoff *et al.*, 1968), but were not found by Seiji & Otaki (Seiji, personal communication). Virus-like particles with similar morphology and arrangement have been found in cisternae of rough endoplasmic reticulum in a number of other transplantable melanomas (Zelickson & Hirsch, 1964; Ito & Mishima, 1967; Epstein & Fukuyama 1970; Balda *et al.*, 1970). The significance of these VLPs and their relationship with the genome of the host cell and with oncogenesis are still at present unknown and are matters of considerable speculation.

ACKNOWLEDGMENTS

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Sarcoidosis in the Bantu

NECROTIZING AND MUTILATING FORMS OF THE DISEASE

J.G.L.MORRISON

Department of Dermatology, University of Pretoria, Pretoria, South Africa

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SUMMARY

Sarcoidosis in the Bantu differs from the same condition in other populations mainly in the severity and the more destructive nature of the lesions. Localization, immune responses and treatment yield similar findings to those met with elsewhere, but necrosis within the granulomata may be prominent and deforming.

Sarcoidosis traditionally shows little or no microscopic sign of necrosis, nor is there much gross mutilation of bones, joints or cartilage. Furthermore, the South African Bantu are claimed to be virtually free from sarcoidosis (Van Lingen, 1961). In our region, none of these statements at present holds true. We have found in Pretoria that, at a rough guess, sarcoidosis is ten times more frequent in the Bantu than in Whites. In a third of the Bantu cases seen here the lesions are destructive and mutilating, such as to allow confusion with leprosy or lupus vulgaris.

Similar trends have been noted in America. Sarcoidosis predominates in the Negro (Michael *et al.*, 1950; James, 1971; Wilkinson, 1972) and there is a tendency towards necrotizing lesions (Klauder, 1925; Sullivan, 1939; Harrell & Durham, 1940; Ronchese & Schiff, 1951; Canizares, 1952; Irgang, 1955). As a highly exceptional feature, necrotizing lesions in sarcoidosis have been reported from Europe (Schaumann, 1924; Simpson, 1963; Bazex *et al.*, 1970).

Our observations are based on a consecutive but somewhat selected series of Bantu patients seen over 9 years. All suffered from gross skin lesions. Some had been admitted to the Westfort Leper Institution, and thence referred to us for investigation and treatment, after leprosy had been excluded. Others had failed to improve on antituberculous and antisyphilitic treatment.

CASE REPORTS

Our series comprised eighteen cases—five men and thirteen women—concentrated between 40 and 49 years of age (seven cases) with an age spread falling off to 13 and 62 years on either side of this peak. The disease had been obvious to all the patients for a few years at least. The skin lesions presented the usual variety of infiltrates met with in sarcoidosis: a lupus pernio-type of distribution (nose, cheeks, hands and fingers) of a papulo-nodular-type, which is unrelated to cold or to season; periorificial papules, nodules and polypoid lesions on the ears, nose and lip margins; papules, nodules and plaques (some psoriasiform) on the head, trunk and limbs; a few lesions in sites of old injury; and

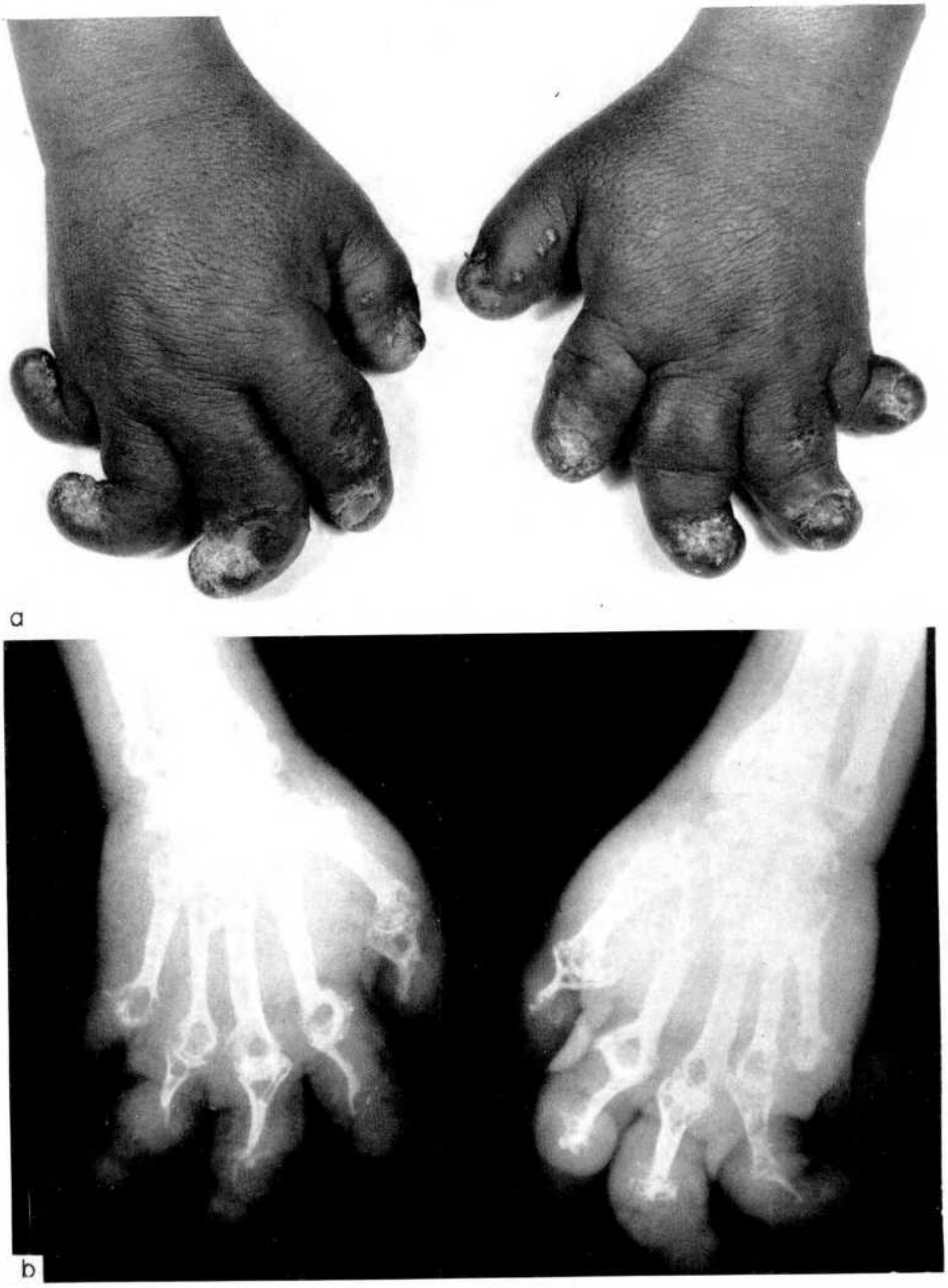

FIGURE I. Diffuse and destructive sarcoidosis of the hands: (a) deformity and nail dystrophy; (b) cystic changes and loss of bone.

nail dystrophies. Two patients had elephantiasis of the legs and feet. Of the eighteen cases, ten showed radiological signs of sarcoidosis in the hands. Two-thirds showed radiological lung changes and one-third had presumptive sarcoidosis of the eyes. Only one case showed lymph gland enlargement and swelling of the parotids. Anergy to intradermal tuberculin was noted in 70%. The diagnosis of tuberculosis was rendered improbable in all our cases by direct sputum examinations, and cultures of sputum and skin tissue, and the lack of response to antituberculous treatment where its trial was considered appropriate. Nine in the series were treated with systemic steroids with a favourable response in all as judged by the skin lesions, although some unsatisfactory features arose. These and other findings represent what may possibly be expected from sarcoidosis anywhere in the world.

What distinguishes our cases is mainly the extent of the changes, e.g. cyst formation with destruction of the joints and disappearance of bone from the hands. In eight cases this had occurred with milder grades of deformity leading to a hand with fingers useless, from want of a skeleton (Fig. 1). In one patient there had been destruction of the nasal cartilages by sarcoidosis in and around the nares (Fig. 2).

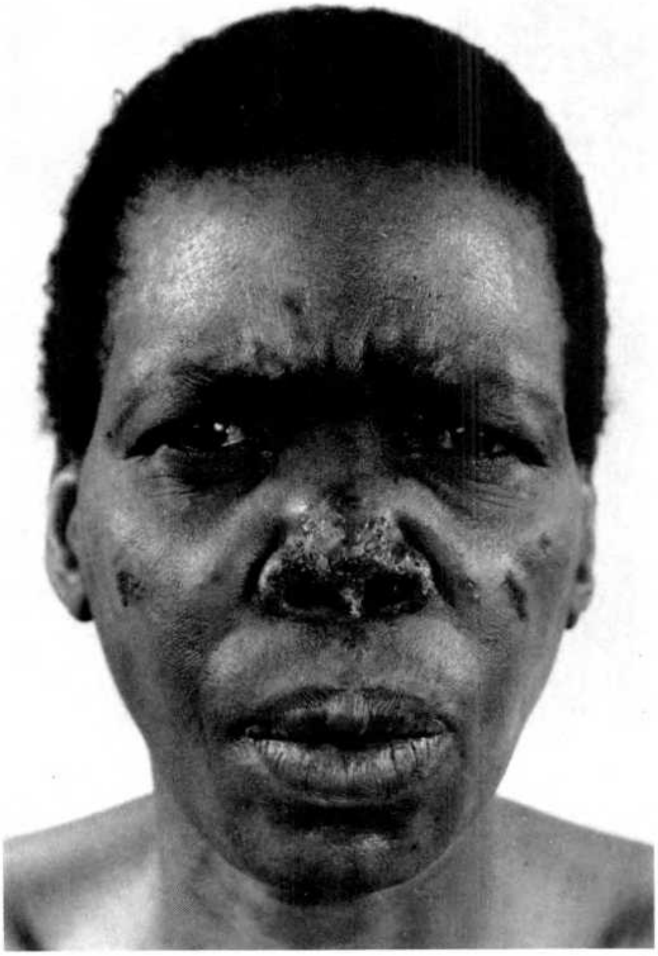

FIGURE 2. Sarcoidosis of the forehead, cheeks and nose. The nose lesions have ulcerated and also resulted in perforation of the nasal septum.

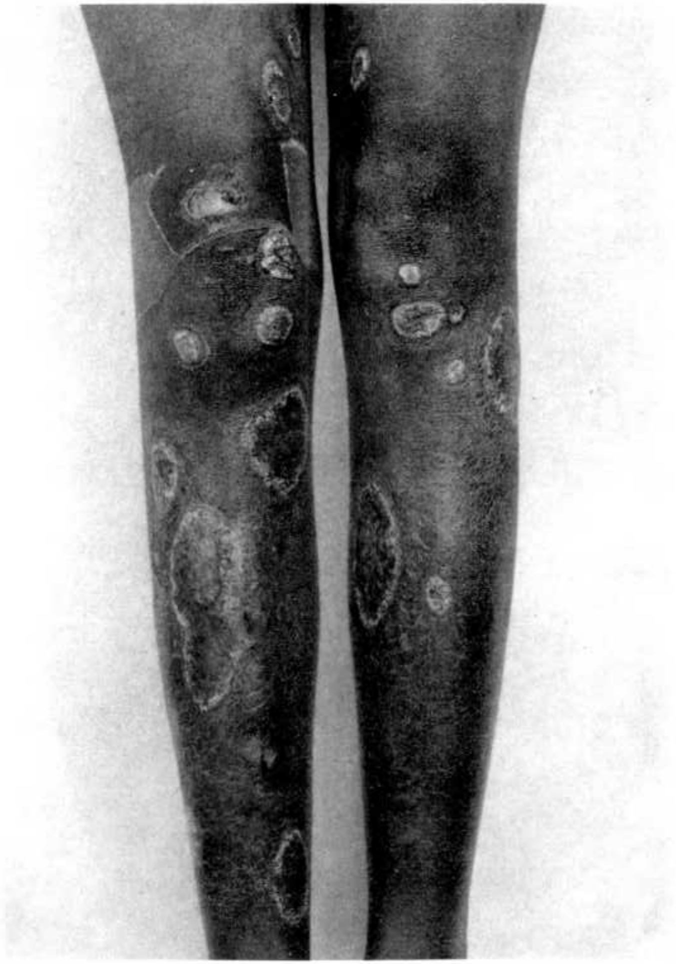

FIGURE 3. Sarcoidosis with psoriasiform, cicatricial and peripherally ulcerating patches on the lower extremities.

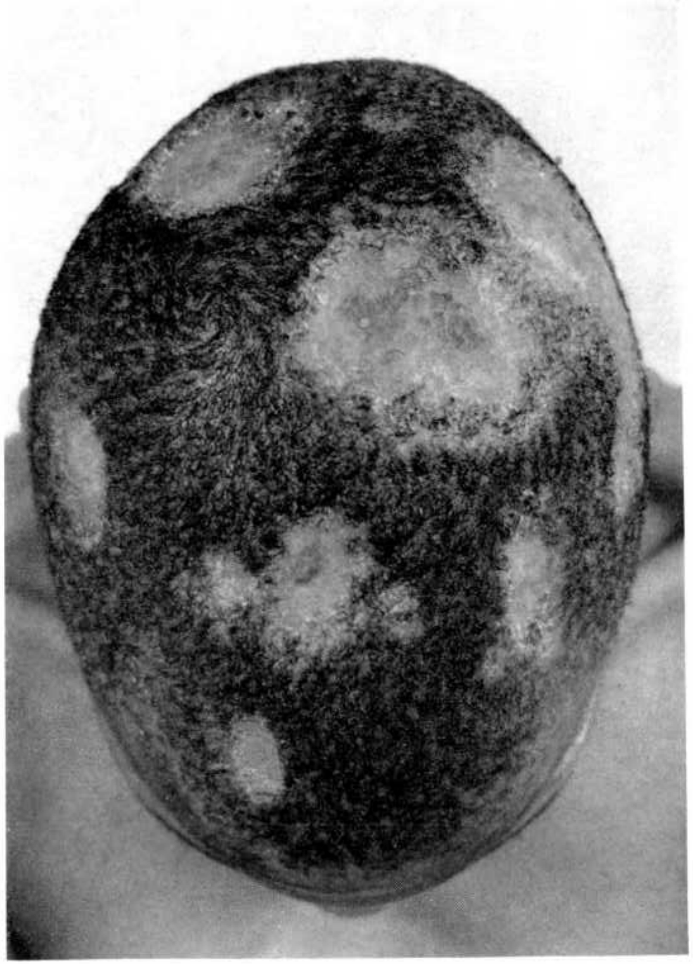

FIGURE 4. Sarcoidosis with cicatricial alopecia.

Similarly, the skin lesions in four cases showed a progression from mere psoriasiform scaling to extensive necrotic crusting, especially at the periphery. This was followed by severe atrophic scarring (Fig. 3). In the scalp, these deep scars showed total loss of hair (Fig. 4). (See also Thomas, 1943; Bluefarb, Szymanski & Rostenberg, 1955.)

Histopathology

In the active phase of the disease, the histology of the skin was as one would expect in sarcoidosis, except in five cases. In these, the epithelioid cells of the sarcoid follicles had undergone fibrinoid

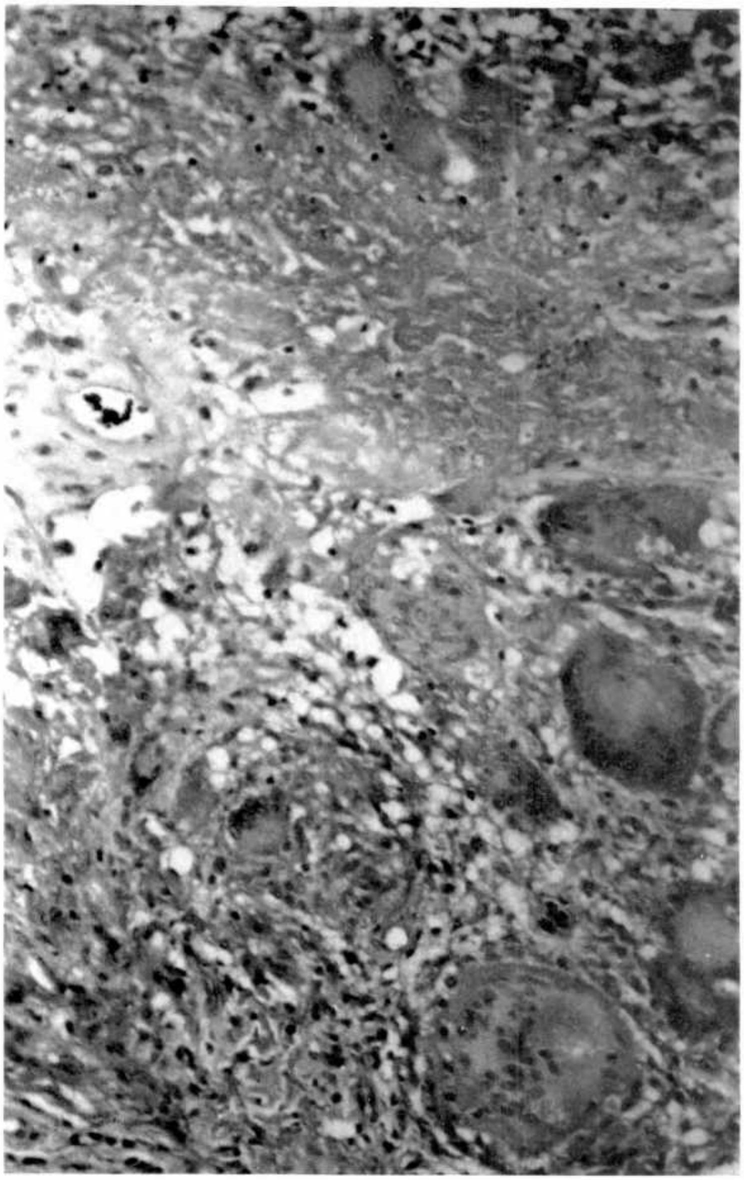

FIGURE 5. Extensive fibrinoid necrosis with surrounding giant cells in necrotizing sarcoidosis.

necrosis, an abundance of Langhans' giant cells was present (Fig. 5), and some of these showed phagocytosis of elastic tissue (Fig. 6), a feature first pointed out in sarcoidosis by Bazex *et al.* (1970). In the healed, atrophic lesions there was merely fibrosis, with no giant cells or elastic tissue. In some places there were remains of epithelioid infiltrates with a few lymphocytes.

We have no tissue available to show if the destructive lesions of the skin are the histological counterpart of the destructive lesions in the fingers. Quite probably they are, but as some of the destructive hand lesions were apparently quiescent, it might have been to little purpose to explore them surgically.

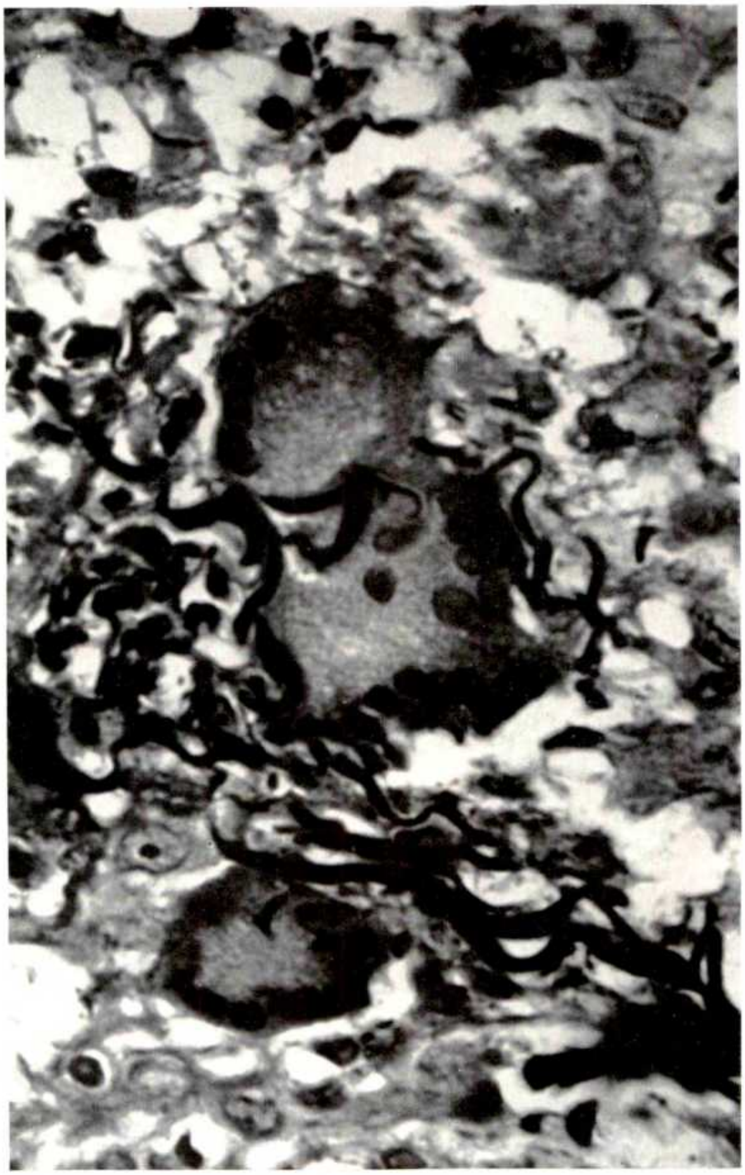

FIGURE 6. Sarcoidosis with elastic fibres engulfed by giant cells.

DISCUSSION

The question still remains unanswered as to why the incidence and severity of sarcoidosis differ among various populations in the world. Could the present-day disease pattern be influenced by some kind of immunological selection? The South African White community has been relatively free of tuberculosis for several generations, while the Bantu are emerging from a scourge of tuberculosis as severe as anything seen in Northern Europe in the late nineteenth century. It seems almost as if severe sarcoidosis in a population follows in the wake of serious tuberculosis.

ACKNOWLEDGMENTS

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The cutaneous reactions produced by dibenzoxazepine (CR)

P.HOLLAND

Medical Division, Chemical Defence Establishment, Porton Down, Wiltshire

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SUMMARY

Dibenz (b.f)-1,4-oxazepine (CR), a substance known to be markedly irritant to skin, has been applied in graded quantities to the skin of volunteers as (a) dry material, and (b) when moistened with saline. The cutaneous reactions produced have been observed and these have been discussed in comparison with those produced by *ω*-chloroacetophenone (CN) and *o*-chlorobenzylidenemalononitrile (CS). CR has been shown to be very potent in causing skin discomfort and pain, equivalent roughly to CN and more so than CS.

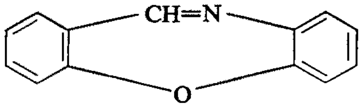
The effects of CR tend to disappear rapidly following its removal but recur, at reduced intensity, when the affected area is washed, even up to 24 h later. The erythema produced by CR fades rapidly and does not recur, whereas that produced by CN and CS remains for a much longer period. Vesication could not be produced with CR, even under adverse and unrealistic conditions. CR is capable of producing acute cutaneous discomfort, apparently out of proportion to the very temporary and moderate changes observed in the skin.

The use of sensory irritant compounds, such as *ω*-chloroacetophenone (CN) and *o*-chlorobenzylidenemalononitrile (CS), for riot control purposes, is well established (Finn, Hogg & Crichton, 1960; Punte, Owens & Gutentag, 1963; Beswick, Holland & Kemp, 1972). When dispersed as aerosols they produce a sensation of irritation in the eyes and respiratory tract to such an extent that exposed subjects are highly motivated to escape from the cloud. In these circumstances the quantity of active material in contact with the skin, which is much less susceptible to sensory stimulation than either eyes or respiratory tract, is so very small that the chances of specifically cutaneous effects being produced are very remote; it is only under exceptionally severe experimental conditions that significant skin reactions have been produced (Holland & White, 1972; Weigand, 1969).

Dibenz(b.f)-1,4-oxazepine (CR) is a very potent stimulator of sensory nerve endings in skin and mucosal surfaces (Ballantyne, Beswick & Price-Thomas, 1973). This property of causing acute discomfort in these structures places CR in the class of compounds which might be chosen for use in military internal operations and therefore the effects of this compound on the skin should be more widely known.

MATERIAL AND METHOD

Dibenz(b.f)-1,4-oxazepine (CR) was prepared at the Chemical Defence Establishment:



Pure
Yellow powder
Melting point, 72°C
Water solubility,
5×10^{-4} (moles/litre)

The subjects were healthy male volunteers, aged between 18 and 30, with no history or clinical evidence of skin disease or allergy. The compound was applied in graded quantities (Table 1) to the skin of the forearm, under a watch glass (4 cm diameter) kept in place with Sleek dressing. Where possible, subjects were investigated in groups of four or six, each group at a different dose level. The material was applied (a) dry and (b) moistened with 2 drops of saline. The duration of contact was 1 h, the substance then being removed with a dry swab, followed by washing with soap and water.

TABLE 1. Numbers of subjects with reactions produced by dibenz (b.f)-1,4 oxazepine (CR) when applied to the skin of the forearm

Compound(mg)	0·5	1·0	2·0	5·0	10·0	15·0	20·0	25·0
CR(dry)	—	—	I−0/2 e+2/2	I+3/6 e+3/6	I+3/6 e+3/6	I+1/2 e+2/2	I+2/2 e+2/2	I+1/6 e+1/6
CR(wet)	I+4/4 e+4/4	I+4/4 e+4/4	I+6/6 e+6/6	I+6/6 e+6/6	I+6/6 e+6/6	I++2/2 e++2/2	I+++2/2 e+2/2	I+++5/6 e++4/6

I, irritation; e, erythema; −, negative; +, mild; ++, moderate; +++, severe.

If a volunteer complained of any undue discomfort during the period of application, the experiment was immediately terminated. The test sites were examined at intervals of 1 h, 6 h, and subsequently daily, for the appearance and development of skin lesions.

RESULTS

Table 1 presents data on the incidence of cutaneous reactions produced by graded amounts of CR, the number of subjects involved and the quantities applied. The cutaneous reactions produced were more marked when the substance was moistened. Irritation was complained of at all dose levels, viz. 0·5 mg and above when moist, but not below 5·0 mg when dry. It appeared within 5 min but disappeared rapidly following removal of the CR. Transient irritation recurred when the affected area was washed up to 24 h later.

Erythema was produced at all dose levels tested (Table 1, Plate 1). It was observed within 10 minutes of application, was not raised and remained localized to the area of application. Following removal of the CR the erythema faded rapidly, and by 15–30 min had disappeared. It never recurred with subsequent washing of the affected area as did the irritation.

No oedema or vesication was produced with any of the dose levels tested. No residual scarring or skin pigmentation was present on inspection of the test sites at 2 weeks following application.

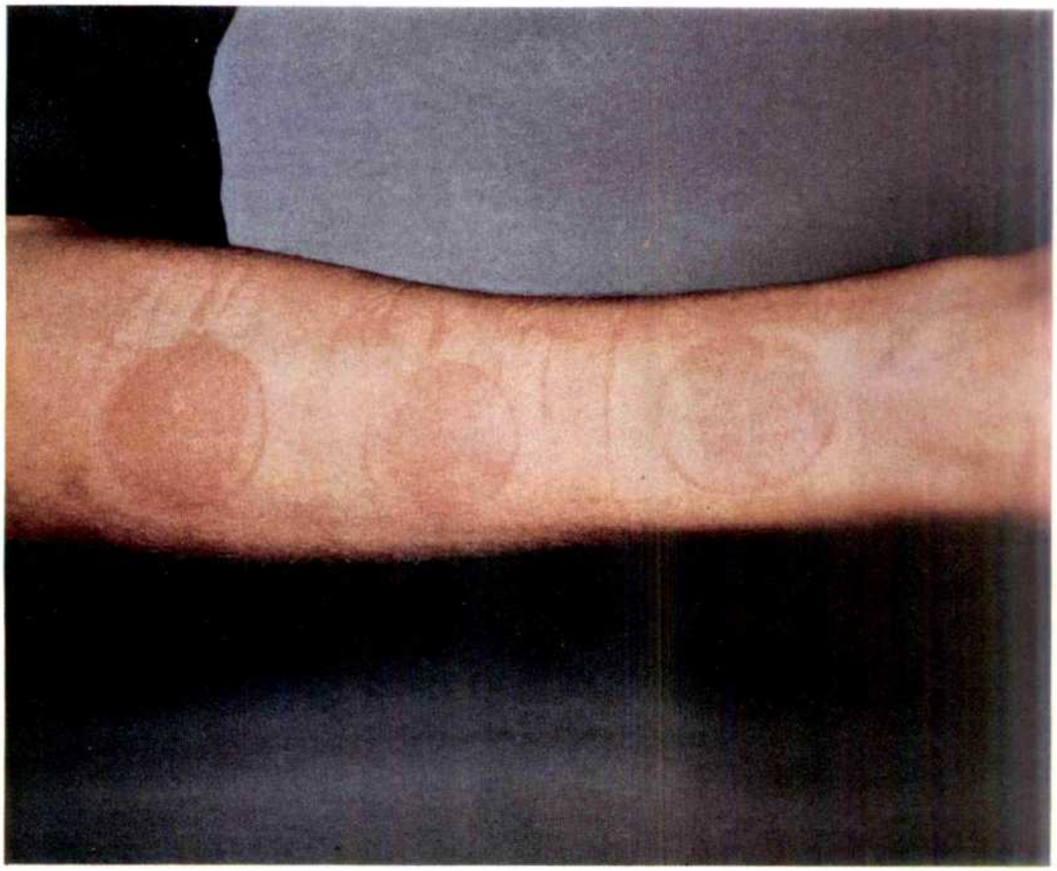


PLATE I. Erythema produced by moistened CR. Left to right: 2·0 mg, 1·0 mg, 0·5 mg.

DISCUSSION

CR has been shown to be a very potent skin irritant. Its irritant potency is greater than that of CS and comparable to that of CN (Holland & White, 1972). When moistened, both CN and CR applied in amounts of 0·5 mg cause marked irritation to be complained of, whereas 10 mg of CS is required to produce the same effect.

The 'tear gas' agent, CN, is capable of producing blisters fairly readily on skin exposed to the material under most conditions. CS on the other hand is only vesicant under very adverse and unrealistic experimental conditions (Weigand, 1969). In the experiment described, CR never produced vesicles even when moistened, in contrast to CN which is capable of producing them even when applied in doses of 0·5 mg.

Erythema produced by CR, although marked, disappeared rapidly after removal of the compound and never recurred with further washing of the affected area. This is in contrast to the erythema produced by CN and CS which, although less intense, may persist over several days.

These limited investigations show CR to be a substance with a high degree of specificity for the pain terminals in skin and, in relation to its potency for causing pain, it produces few of the classical signs of inflammation, with the exception of an erythema which remains circumscribed. The local effects observed in the volunteers studied in this series were such that, apart from thorough decontamination of the affected part, no specific treatment was considered to be necessary.

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Pharmacology and Treatment

Methotrexate induced liver disease in psoriasis

G.H.MILLWARD-SADLER AND T.J.RYAN

Departments of Pathology and Dermatology, United Oxford Hospitals

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SUMMARY

A review of the effects of folic acid antagonists in psoriasis has now been completed. All nineteen patients have had such therapy for at least 1 year and some for up to 10 years. Seventeen of these patients have had recent liver biopsies and another two were studied at post mortem. The biopsy findings were analysed according to mode(s) of therapy. All three patients with cirrhosis and three of four with fibrosis had received significant amounts of oral methotrexate. Eight patients had received methotrexate only by parenteral injection and only one biopsy showed mild fibrosis. A possible ancillary factor is the severity of psoriasis.

Methotrexate (MTX) now seems established as a factor causing or contributing to hepatic fibrosis and cirrhosis in patients with psoriasis (O'Rourke & Eckert, 1964; Coe & Bull, 1968; Weinstein *et al.*, 1970; Roenigk *et al.*, 1971; Dahl, Gregory & Scheuer, 1971, 1972; Almeyda, Barnado & Baker, 1971).

Many of the more recent reports demonstrate an association with alcohol (Almeyda *et al.*, 1971; Weinstein *et al.*, 1970; Roenigk *et al.*, 1971), whereas Dahl *et al.* (1972) suggest that the dose, frequency and duration of therapy with MTX are more important factors.

Ryan *et al.* (1964) were the first in this country to emphasize the importance of liver function tests when monitoring patients on folic acid antagonists. Some of the psoriatic patients included in this survey have been on this drug since. We present here our findings in these and subsequent patients with psoriasis, who have received significant amounts of MTX for a longer period than that reported in other series.

THERAPEUTIC REGIMES

Patients receive MTX either orally or parenterally and these routes reflect qualitative differences.

Oral therapy

This method employs 2·5 mg/day for a number of consecutive days varying according to clinical response but usually between 7 and 12 days. The remainder of a 2-week schedule is used as a rest period.

Parenteral therapy

Intravenous or intramuscular doses of 25–50 mg each are given at intervals of 2–4 weeks, rarely longer. For the last few years only the intramuscular route has been used.

Both regimes

In some instances in the course of the last 10 years both methods have been employed for the same patient. If less than three-quarters of the total therapy has been given by one method then the patient is placed in this group.

PATIENTS

Of our patients on MTX for psoriasis we have a group of seventeen who have both received MTX for more than 1 year and had recent liver biopsies. In addition there are two patients on whom we have only post mortem histology.

Age and sex

The age and sex of the seventeen patients are shown in Table 1. There are six men, average age 41 years, and eleven women, average age 60 years. Because of this discrepancy, results are analysed according to sex. The two patients studied post mortem were males aged 85 and 48.

Associated conditions

Grade of psoriasis. The severity of the psoriasis has been graded as mild, moderate and severe according to the surface area involved, acuteness of onset, virulence, and complications, particularly arthropathy.

Diabetes mellitus. Three patients, two females and one male, have diabetes. The man has since died from a cardiovascular accident due to atherosclerosis. At autopsy, the liver, after 2½ years of intramuscular therapy, was unremarkable and histology showed mild fatty change with no fibrosis.

Alcohol. No attempt has been made to exclude heavy drinkers or alcoholics although all patients have been carefully questioned. In only one case may alcohol be significant; this is a man who admits to drinking 30 pints of beer per week.

Others. One female has a mucocoele of the gall bladder and another female has had recurrent papillomata of the bladder for 8 years. A man has recently died from coronary thrombosis due to atherosclerosis. No fibrosis of the liver was found at autopsy although he had had 6½ years of intramuscular therapy.

Two men were studied only at post mortem. One died during fulminating generalized pustular psoriasis, possibly associated with septicaemia, and the other of carcinomatosis arising in the sigmoid colon.

Liver biopsy

Each patient had a percutaneous liver biopsy using a Klatskin needle. The specimen was fixed in formalin and processed in a routine manner. Sections were cut at 5 μm and stained as follows: haematoxylin and eosin; reticulin (Gordon and Sweet); Perl's Prussian Blue; periodic acid Schiff; diastase/periodic acid Schiff; MSB or Goldner Trichrome.

Two patients had liver biopsies in 1964. These and the present biopsies have been reviewed without knowledge of the therapy.

RESULTS

The two biopsies from 1964 show minor abnormalities and no fibrosis. In the seventeen recent biopsies there are five showing no abnormality, seven showing minor abnormalities, two showing fibrosis and three showing cirrhosis. 'Minor abnormalities' are also present in these last two groups.

No abnormality

There are three men and two women. Four patients had not received MTX for at least 6 months before the biopsy although duration of treatment ranged from 18 to 54 months. The fifth patient is male and has received all MTX as oral therapy for 8½ years without a break.

Minor changes

These only are seen in seven biopsies and vary slightly. Mild, randomly distributed fatty change is found in six, and small foci of necrosis in centrilobular areas in five biopsies. These foci are composed of one or two degenerate hepatocytes surrounded by mononuclear cells. Occasional changes include vacuolation of hepatocyte nuclei in three biopsies and slight oedema in portal tracts in two biopsies. When present any inflammatory infiltrate is scanty and mixed in character. The two biopsies from 1964 also fall into this category.

Fibrosis

The two cases of fibrosis are a female on parenteral MTX who also has a mucocoele of gall bladder and a male on oral therapy for 6½ years.

In both biopsies the fibrosis is mild, and is seen as short slender spurs enlarging portal tracts. The biopsy from the female shows small proliferating bile ductules, particularly within these spurs. It is similar to the changes described by Poulsen & Christoffersen (1970) in an analysis of liver biopsies from patients with disorders of the biliary tree.

Both biopsies also show fatty change and a few foci of necrosis; neither shows significant distortion of the lobular architecture.

Both autopsied patients show, on post mortem histology, severe fibrosis of the liver sufficient to produce some linking of portal tracts.

Cirrhosis

Three biopsies show a micronodular cirrhosis; all are from female patients. Of these patients, two have diabetes mellitus and one of these was ill for 12 months prior to biopsy with a history of ankle swelling and shortness of breath. The third female was apparently well apart from her psoriasis. In all three patients further investigations, such as for antinuclear factor and Australia antigen, failed to reveal any other incriminating aetiology.

The biopsies show extensive dissection by areas of collapse and fine fibrosis; the intervening parenchyma shows fatty change as well as pleomorphism of cells and nuclei. Twinning of cell plates and nodular regeneration are identified. The inflammatory infiltrate is minimal and composed of macrophages, lymphocytes and neutrophils with a few plasma cells. Some piecemeal necrosis is present.

In one biopsy these changes are particularly florid and the variation of nuclear size considerable. The larger nuclei are sometimes polypoid and hyperchromatic but most have large vacuoles in the nucleoplasm. On electron microscopy these prove to be invaginations of cytoplasm.

It is noteworthy that one of these females had a liver biopsy in 1964 which shows mild fatty change only.

Relationship to therapy (*Table* 1)

Parenteral therapy. Eight patients (six females, two males) received parenteral MTX for between 1

TABLE 1. Age, sex and relationship of mode of therapy to biopsy result

		Duration of therapy (months)			
Sex	Age (years)	Parenteral	Oral	Total	Liver biopsy result
Male	26	12	—	12	Normal
	57	78	—	78	Normal
	63	12	18	30	Minor changes
	36	36	39	75	Minor changes
	23	12	69	81	Fibrosis
	43	—	102	102	Normal
	48	6	18	24	PM only: fibrosis
	85	36	48	84	PM only: fibrosis
Female	54	12	—	12	Minor changes
	73	36	—	36	Minor changes
	43	57	—	57	Normal
	68	66	—	66	Fibrosis
	61	87	—	87	Minor changes
	84	102	—	102	Minor changes
	57	24	12	36	Minor changes
	71	30	66	96	Cirrhosis
	28	—	36	36	Normal
	68	—	48	48	Cirrhosis
	51	—	102	102	Cirrhosis

and 8½ years. Five of these were treated for 5 years or more. None has cirrhosis and only one shows fibrosis. This patient is the female with a mucocoele of the gall bladder who has had MTX for 5½ years.

Oral therapy. Five patients (three females, two males) received oral MTX for periods between 3 and 8½ years; two females have cirrhosis and one male shows fibrosis.

The two females have received MTX continuously for 4 years and 8½ years. The third unaffected female has had MTX for 3 years but this has been broken up into four periods of 9 months each, separated by intervals of 3 months.

The man with mild fibrosis of the liver has had parenteral MTX for 1 year, followed by oral therapy for 5½ years. The striking exception is the male treated with MTX for 8½ years by the oral route. No fibrosis is seen in his liver biopsy.

Mixed therapy. Six patients (two females and four males) have received both parenteral therapy and oral therapy as described. Four of these have had biopsies. One biopsy shows cirrhosis. This is in a female who has received MTX for a total of 8 years and for the last 5½ years has followed an oral regime. The other three biopsies show minor changes. Of interest in this group is the male who drinks 30 pints of beer per week and has had 3 years parenteral MTX followed by 2½ years oral MTX. The remaining two biopsies are from patients treated for 2½ years and 3 years.

The two men studied only at autopsy fall into this group. One had been treated for 2 years including oral therapy for 18 months and the other for 7 years including oral therapy for the first 4 years.

Grading of psoriasis (*Table* 2)

Severe psoriasis is or was present in eight patients (four females and four males), including the two

TABLE 2. Grade of psoriasis related to mode of therapy and eventual outcome

Psoriasis	Total	Cirrhosis	Fibrosis	Unremarkable
Severe				
Oral*	5	2	2	1
Parenteral	3	0	0	3
Moderate				
Oral*	3	1	1	1
Parenteral	4	0	0	4
Mild				
Oral*	3	0	0	3
Parenteral	1	0	1	0

* The mixed group has been included under 'oral' for this table.

men who had no liver biopsies. Two of the females have cirrhosis, two males have fibrosis, and the remaining four have only minor changes in their biopsies. It is of interest that the two females with cirrhosis and the two males with fibrosis are all patients in a group who have received significant oral therapy. The sole remaining patient in this group had had therapy for the shortest period—18 months.

A moderate degree of psoriasis is present in seven patients (five females and two males). One female has cirrhosis, and one male fibrosis. Both these are on oral therapy: one other female receives treatment orally.

Four patients have mild psoriasis (two females and two males). One female receives MTX parenterally and has mild fibrosis of the liver. The other three have received MTX, if not entirely, at least for prolonged periods, by the oral route. None has fibrosis or cirrhosis of the liver.

DISCUSSION

Ryan *et al.* (1969) reported that liver function tests are often abnormal during the first year or so of therapy with the folic acid antagonists. However, neither then nor retrospectively is there any correlation between the abnormal liver function tests and the development of fibrosis or cirrhosis. We find our results support the contention by Dahl *et al.* (1972) that MTX when administered by a continuous low dosage (oral) regime has more serious sequelae than when given by an intermittent high dosage regime. All three patients with cirrhosis had had significant amounts of MTX orally. Only one patient in the parenteral therapy group showed fibrosis, which was mild and was also open to an alternative explanation.

The difference in toxicity between these two routes of administration is not well understood, but it seems significant that Condit (1960) was able to demonstrate reduction in human urinary citrovorum factor for at least 2 months after a 13-day course of oral MTX, compared with 2–4 weeks after a single intravenous dose of equivalent amount. Johns *et al.* (1964), who injected labelled MTX intravenously, suggested that its rate of entry into the liver cells was slow and that none was circulating after 48 h. Continuous low dosage therapy would provide free plasma levels for much longer periods and may conceivably result in a greater percentage uptake by hepatocytes.

We do not find fibrosis an inevitable accompaniment of oral therapy as shown by our male patient, who has successfully survived oral treatment for 8½ years. This patient has had the least aggressive therapy in the group, usually taking tablets for only 7 out of the 14 days. His psoriasis is predominantly acral and elsewhere is mild.

It seems probable that ancillary factors may potentiate the drug hepatotoxicity. We have noted that all three patients with cirrhosis are female.

It has been shown that severe generalized pustular psoriasis is more likely to occur in elderly females (Ryan & Baker, 1971), whereas males are more likely to be given MTX if psoriasis threatens their job or career. This could produce a discrepancy in the severity of the psoriasis between the sexes that may account for the preponderance of cirrhosis in the females of our oral therapy group.

Zachariae & Schiodt (1971) suggested that the severity of psoriasis may influence the liver biopsy findings. The highest frequency of abnormal liver biopsies was in patients with psoriatic erythroderma. None of their treated patients had fibrosis; all had received parenteral MTX. We wondered if this may be an ancillary factor affecting our results, for it is intriguing that in the oral and mixed therapy groups eight patients have moderate to severe psoriasis, of whom three have cirrhosis and three fibrosis.

In contrast, three patients in the oral group have mild psoriasis and none has significant fibrosis on liver biopsy.

Whilst the evidence is not conclusive, we believe that there may be increased MTX toxicity in patients with severe psoriasis. Review of the literature is unhelpful, as age, sex, mode of therapy and grade of psoriasis are not simultaneously defined.

Two of the three patients with diabetes mellitus have cirrhosis. There is a vague and ill-defined relationship between these two conditions and analysis of the problem is complicated by the abnormal glucose metabolism found in cases of cirrhosis. We have no clinical evidence of an excessive alcohol intake in any patient with an abnormal biopsy, nor have we seen the stigmata of acute alcoholic hepatitis in these biopsies. We cannot believe that alcohol is necessary even as an adjunct to MTX in the aetiology of this cirrhosis, as suggested by Almeyda *et al.* (1971), though we do not deny its possible effects.

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Distribution of methotrexate in rat tissues

P.C.H.NEWBOLD

Department of Medicine, Postgraduate Medical School, Hills Road, Cambridge CB2 2QL

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SUMMARY

The distribution of methotrexate between tissues is a very complex process, dependent in part upon the species studied, the size of the dose and the channel of administration. The nature of the tissue and its content of dihydrofolate reductase also play a part. Elimination of methotrexate in the urine and bile indicate that renal and hepatic function are also important.

The amounts of administered methotrexate taken up by the skin and liver were doubled by experimental folate depletion. In contrast, folate deficiency did not significantly increase uptake by the kidneys or intestines. The skin uptake of methotrexate was increased about six-fold by prior stripping, without influencing the hepatic uptake. Methotrexate taken up could be partially displaced by pteridines which have affinity for dihydrofolate reductase. Methotrexate itself was most efficient, but both dihydrofolate and folic acid were more effective than leucovorin, while 5-methyl folate had virtually no effect.

These results indicate that a risk of hepatic injury is probably inseparable from the therapeutic action of methotrexate in psoriasis, even though the amount of drug reaching the liver can be reduced by using a logical parenteral schedule.

The distribution of methotrexate (MTX) between tissues is a complex and multifactorial process. Nevertheless, models have been constructed, based upon the factors listed in Table 1 (Bischoff, Dedrick & Zaharko, 1970; Werkheiser, 1971; Zaharko *et al.*, 1971). This paper refers to experimental administration of MTX to rats, in an effort to quantify the uptake by the tissues. As a background to this study, biochemical parameters were established for tissue protein, DNA and dihydrofolate reductase (DHFR). These are listed in Table 2. In subsequent experiments, the effects of strip-

TABLE 1. Factors influencing tissue distribution of methotrexate

Species	Channel of administration
Size of dose	Serum protein binding
Renal function	Length of exposure
Organ, and size	Arterial supply
Intestinal absorption	Biliary excretion
DHFR content	Folate status

ping of skin upon the uptake of MTX were measured, in an attempt to simulate the increased activity which occurs in psoriasis. The effects of folate deprivation on MTX uptake were also studied by inducing folate deficiency. This state was confirmed by measuring the uptake of labelled folic acid in control and depleted animals. The folate-depleted rats were also used to investigate the effect of folate deficiency on MTX uptake. Finally, the displacement of labelled MTX from the tissues was studied, using cold MTX and other pteridines.

MATERIALS AND METHODS

Sprague–Dawley rats were used throughout, and they were given an unlimited diet except where specified. Tissues were removed quickly, and either stored frozen, or homogenized in buffer as already described (Newbold, 1973). Protein was measured by the biuret method, and DNA by the diphenylamine method (Burton, 1956). DHFR was assayed spectrophotometrically as previously reported (Newbold & Stoughton, 1972). The liquid scintillation mixture was composed of 5 g of butyl-PBD dissolved in 100 ml of Bio-solv BBS2 and 900 ml of toluene. This gave a basic counting efficiency of 35% for tritium. Tritiated MTX (3′-5′-Na salt) had specific activity of 250 mCi/mM, and ^{14}C-labelled folic acid (2-C^{14}, K salt) also had specific activity of 250 mCi/mM, and both were obtained from Amersham–Searle. These were both checked for purity by chromatography before use, and old material was not used because of its instability in storage.

TABLE 2. Protein, DNA, and DHFR content of young rat tissues

Tissue	% age wt.	Protein (mg/g)	DNA (mg/g)	DHFR (U/g)	DHFR (U/g protein)	DHFR (U/mg DNA)
Skin	9·5	131·46	0·274	4·35	33·1	15·88
Liver	4·6	148·47	0·577	1·14	7 68	1·98
Kidney	1·0	160·27	1·77	4·13	25·77	2·33
Intestine	2·0	151·60	0 44	0·556	3·67	1·26
Spleen	0·3	316·5	5·875	3·75	11·85	0·64
Brain	3·0	111·55	0·392	0 245	2·20	0 63

RESULTS

As a background to work with rats, some biochemical and tissue parameters were established. Table 2 summarizes the results which are derived from the average of twenty 3-day-old rats of about 10 g weight. The skin, which included the dermis, gave a higher value for DHFR activity than the liver. As the fur is about to erupt, and as they have not yet begun to eat normally, this would seem to be a normal finding. Although the brain needs folate coenzymes, it is not known to contain DHFR. So the observed levels are probably due to the blood contained in the samples; at least in the rabbit, the choroid plexus seems able to actively exclude MTX (Rubin, Owens & Rall, 1968), so avid uptake would not be expected.

Uptake of labelled MTX

Another group of 6-week-old rats was given intraperitoneal injections of ^{3}H-MTX, and killed after either 2 or 8 h. Table 3 summarizes the results of counting aliquots of the tissues homogenized, in the standard scintillation fluid. In all cases, counts per minute are expressed per mg wet weight of tissue. Co-chromatography was carried out to confirm that the radioactivity measured was still MTX.

TABLE 3. Uptake of tritiated methotrexate by tissues of 6-week-old rats

		Tissue				
Dose (μg)	Time (h)	Skin	Liver	Intestine	Kidneys	Brain
25	2	241 (42)*	6355 (203)	1343 (116)	59 (12)	53 (12)
	8	753 (90)	9540 (511)	2933 (173)	9162 (400)	358 (37)
50	2	95 (20)	10160 (1254)	3142 (437)	13090 (1167)	48 (10)
	8	272 (33)	10758 (1693)	2540 (891)	11915 (2319)	93 (14)
100	2	123 (18)	11650 (2517)	3560 (512)	16230 (2853)	65 (11)
	8	631 (72)	11102 (1641)	2663 (374)	11461 (1827)	177 (28)

*Standard deviations are given in brackets.

The counting efficiency was 35% for skin, 33% for brain, 31% for intestine, 28% for kidney, and 25% for liver—the lower figures being due to the inevitable colour and chemical quenching. 'Intestine' refers to small intestine, without the contents. The kidneys were homogenized in pairs from each animal, and the brain was well washed with three changes of ice-cold *tris*-HCl buffer, pH 7·5, to remove as much blood as possible. Fur-bearing skin was shaved before the experiments, so that homogenization was possible. Each figure is the average of at least three samples from each of four rats. These results confirm that the kidney receives a large portion of MTX after parenteral injections. However, the importance of excretion by the kidney cannot be confirmed, as no studies on urine were included in these experiments. Tissue uptake is not simply related to dose administered, and the amount reaching the skin is less than might have been expected in terms of its metabolic activity.

Effects of stripping the skin

These results were contrasted with a matched group of 6-week-old rats, in which the backs were closely shaved. The skin was then stripped successively with cellulose tape until the whole area was glistening and oozing. This took between five and eight stripping steps. After 48 h, MTX was injected, and the animals killed after 2 or 8 h as before. Only the skin which had been injured was used for assays, and then only if it was not infected. Table 4 shows that there was on average a six-fold increase

TABLE 4. Uptake of ^{3}H-methotrexate after stripping of skin of back

		Tissue		
Dose (μg)	Time (h)	Skin	Liver	Kidneys
25	2	1350 (112)*	5724 (490)	143 (39)
	8	4809 (183)	6539 (713)	6720 (980)
50	2	1500 (127)	8527 (1125)	5372 (1352)
	8	5134 (196)	9370 (1602)	7042 (1219)
100	2	1872 (142)	8918 (906)	9250 (537)
	8	5263 (212)	9745 (1538)	15369 (1608)

*Standard deviations are given in brackets.

TABLE 5. Uptake of ^{14}C-folic acid

		Tissue				
	Time (h)	Skin	Intestine	Liver	Kidneys	Brain
Controls	2	19·5 (2·5)*	314 (36)	733 (102)	3705 (341)	156 (13)
	8	84 (11)	905 (94)	618 (127)	473 (28)	14 (2)
Folate-depleted	2	140 (11)	410 (26)	1136 (138)	930 (49)	380 (30)
	8	530 (19)	1223 (114)	2408 (209)	568 (28)	337 (32)

*Standard deviations are given in brackets.

in the skin uptake of MTX. Although this might be taken to indicate a closer approximation to what is happening in psoriasis, it is noteworthy that the liver uptake is not greatly changed, so that the risk of hepatic damage is not reduced. Here again, the counts per minute are expressed per mg wet weight of tissue, and each point is the average of at least twelve samples.

Effect of folate depletion

To investigate the effects of folate depletion, an attempt was made to induce folate deficiency in some matched litters. Throughout pregnancy the mothers were fed 5 mg of neomycin elixir daily, had drinking water which contained neomycin 1 mg/100 ml, and were kept in cages with wire bottoms so that the faeces fell through and could not be consumed. Daily attempts were made to feed them with 2 mg of neomycin elixir via pipettes—to sterilise the intestinal contents. The aim was to combine dietary restriction of folate with the prevention of coprophagy, as the faeces contain significant amounts of folate. It is realized that this is an imperfect method, but if MTX were used in the diet, the result would have been lower folate levels, but it would have defeated the point of the study. Table 5 contrasts the uptake of 13.0 μg of ^{14}C-folic acid in control and in folate-depleted rats, of 6 weeks of age. Co-chromatography was carried out to check that the radioactivity measured was still folic acid and not a metabolite. Tissues were removed quickly, homogenized cold, and aliquots counted in the standard scintillation fluid. Efficiency of counting was 53% for liver, 47% for kidney, 62% for intestine and 67% for brain and for skin. These results suggest that folate deficiency had in fact been produced, as shown by the greater uptake by skin, liver and brain, and the reduced uptake by the kidney. Statistical analysis of these data gave a P value of 0·001. Again, counts per minute are expressed per mg wet weight of tissue. Each figure is derived from samples from at least eight rats.

Supported by these results, the uptake of ^{3}H-MTX was studied in other litters of 6-week-old

TABLE 6. Uptake of ^{3}H-methotrexate in folate-depleted rats

		Tissue			
Dose (μg)	Time (h)	Skin	Intestine	Liver	Kidneys
50	2	132 (16)*	4238 (223)	12530 (823)	5830 (279)
	8	456 (21)	1972 (174)	16120 (1120)	7314 (628)
100	2	205 (19)	5083 (916)	12178 (970)	6387 (510)
	8	712 (28)	3127 (480)	12436 (1436)	5762 (464)

*Standard deviations are given in brackets.

folate-depleted rats. These were closely similar in weight to those animals used for the experiments listed in Table 3. The results are shown in Table 6, where counts per minute are again expressed per mg wet weight of tissue. Each figure is derived from samples from five rats. These results indicate that although folate depletion does increase the uptake of ^{3}H-MTX, it is by a very much smaller amount than the uptake of ^{14}C-folic acid referred to above. This agrees with the evidence that MTX and folic acid do not share the same active uptake mechanism. The results also provide a basis for the increased toxicity of MTX noted in patients with cancer, many of whom are folate-depleted (Hellman, Iannotti & Bertino, 1964).

Displacement studies

To investigate the displacement of MTX, several matched litters of 6-week-old rats were all given 100 μg of ^{3}H-MTX intra-peritoneally. After 2 weeks, the controls received injections of sterile water, and groups of five rats were given, intra-peritoneally, a single injection of 1 mg of the displacing agent dissolved in buffer. All animals were killed 24 h later, by ether anaesthesia and cervical dislocation. Tissues were removed and counted as above. The residual radioactivity, as counts per minute per mg wet weight of tissue, was compared with the controls; the percentages of stored MTX displaced are shown in Table 7.

TABLE 7. Displacement of ^{3}H-methotrexate

	Tissue			
Drug	Skin	Liver	Intestine	Kidneys
Methotrexate	28·4 (2·2)*	75·0 (11)	12·1 (0·6)	31·2 (5·3)
Dihydrofolate	17·3 (1·5)	16·1 (1·7)	7·3 (0·5)	9·4 (2·7)
Folic acid	8·6 (0·8)	4·7 (0·9)	2·5 (0·3)	5·1 (1·2)
Leucovorin	3·1 (0·3)	1·0 (0·1)	1·2 (0·2)	1 6 (0·4)
5-methyl folate	0 6 (0·1)	0 9 (0·2)	0·3 (0·2)	0·7 (0 2)

* Standard deviations are given in brackets.

These results accord with earlier work (Johns *et al.*, 1964b), allowing for the differences in dose and species. The effect of species has been demonstrated with cultures of various neoplastic cells (Harrap *et al.*, 1971); so it would be unwise to project from this animal work any hypothesis about human conditions. However, the results also accord with studies of human lymphocytes and L 1210 cells that more than one uptake mechanism is involved (Niethammer & Huennekens, 1973; Huennekens *et al.*, 1973). The use of leucovorin in conjunction with MTX has been recommended for treating psoriatics (Ive & De Saram, 1970), although this does entail the risk of a relapse (Cipriano, Selsky & Bertino, 1970). Table 7 suggests, if humans behave like rats in this respect, that administering folic acid or dihydrofolate may be a more efficient means of displacing bound MTX. This suggests another possible treatment.

Ideally, a psoriasis-DHFR-specific inhibitor would be required (Comaish, 1969), but it is unlikely that such a compound exists, because there is as yet no evidence to suggest that the enzyme is qualitatively abnormal in psoriasis. Alternatively, a flushing-out process, favouring organs other than the skin, could displace unwanted MTX from where it does harm. This would leave a therapeutic level of MTX where it was required.

DISCUSSION

The importance of species is clearly illustrated by the guinea-pig and the rabbit, which are both resistant to the action of anti-folates (Girdwood, 1951), and do not show increase in hepatic DHFR when given repeated small doses of MTX (Bertino, Simmons & Donohue, 1962). However, the purified enzyme is sensitive to MTX *in vitro*, and the mechanism of resistance is now known to be oxidation of MTX to an inactive form (Johns & Valerino, 1971). It is likely that most other inter-species differences in sensitivity to MTX can be explained by differences in distribution and clearances (Zaharko *et al.*, 1971), which will influence access to the target DHFR. Present evidence is that each animal has the same enzyme in all of its tissues— as shown in the guinea-pig (Bertino *et al.*, 1966), and in humans (Anderson *et al.*, 1970; Bertino, Cashmore & Hillcoat, 1970).

The channel of administration has important effects. MTX was reported to be rapidly and completely absorbed if given by mouth (Freeman, 1958), yet other reports indicate that absorption can be saturated, and becomes incomplete at higher doses (Henderson *et al.*, 1965a; Henderson, Adamson & Oliverio, 1965b). Consequently, the channels of elimination will vary in importance. After an oral dose of MTX, 30% appears in the urine and 70% in the stool (Henderson *et al.*, 1965b). By contrast, injected MTX is eliminated mainly in the urine. After a single injection, up to 40% can appear in the urine in 5 h (Liguori *et al.*, 1962), up to 50% in 8 h (Johns *et al.*, 1964a), 'the bulk' in 6–8 h (Patel *et al.*, 1969), or 'up to 99%' in 12 h (Freeman, 1958). Clearly the total dose can have some effect (Condit, 1960a; Holland, Purdy & Wilson, 1968). Nevertheless, the amount of MTX stored in humans and mice is not clearly related to the dose (Charache, Condit & Humphreys, 1960).

The effect of MTX differs according to the rate of cell division (Hryniuk, Fischer & Bertino, 1969; Hryniuk, 1972), but the tissue uptake has been shown not to be related to mitotic activity (Patel *et al.*, 1969). The degree of serum protein binding of MTX can be high (Freeman, 1958), and this may be a significant variable between species and between individuals. The binding of folic acid has been shown to be constant over a wide range of concentrations (Johns, Sperti & Burgen, 1961), and the close similarity of structure between MTX and folic acid makes it likely that this is also true for MTX. The length of exposure to MTX is important, as higher tissue levels are seen with prolonged courses and with infusions, so the arterial supply of an organ is significant (Anderson *et al.*, 1970). The liver, kidney, stomach, and gonads have been reported to contain more MTX than other organs of comparable size (Johns *et al.*, 1961); while in human cases coming to autopsy, highest levels have been found in kidney, gall-bladder, liver, skin, and small intestine (Anderson *et al.*, 1970).

Storage of MTX is said to be mainly in the liver (Henderson *et al.*, 1965a, b), but it has been found that MTX persists in mouse kidney for more than 100 days (Charache *et al.*, 1960). The liver MTX levels may partially reflect high concentrations in the bile, as the biliary secretion rate is important (Oliverio & Zaharko, 1971). The amounts secreted into the bile, and re-absorbed by the gut, mean that multiple cycles can be established, if intestinal absorption is adequate. In mice, labelled MTX has been shown to persist in intestine as well as in kidney and liver (Darzynkiewicz *et al.*, 1966). Chronic administration of MTX to rats also leads to storage in the intestine (Newbold, 1973). The bound MTX can be slowly converted to forms with lesser affinity for DHFR (Johns *et al.*, 1964b), or metabolized (Condit & Yoshino, 1968). The amount of MTX taken up by a tissue has been related directly to the drug-combining capacity of its contained DHFR (Werkheiser, 1962). It is well known that the tightness of binding of MTX is reduced if the pH is raised above 7·5 (Bertino *et al.*, 1964; Bertino & Hillcoat, 1968). Perhaps the observed dissociation of the enzyme-inhibitor complex may be upon this basis (Stanley, Neal & Williams, 1969).

MTX taken up can be displaced by compounds with affinity for DHFR. Two weeks after an injection of labelled MTX, displacement is most efficient with cold MTX, but it is significant also with dihydrofolate and folic acid, and measurable with Leucovorin and methyl folate (Johns *et al.*, 1964a). Uptake of MTX into certain sarcoma and leukaemia cells can be markedly inhibited by 5-methyl tetrahydrofolate and 5-formyl tetrahydrofolate (Leucovorin), but not by folic acid, dihydrofolate, or 10-formyl folic acid (Harrap *et al.*, 1971). In other tissues, influx of MTX can be competitively inhibited by folic acid and Leucovorin (Lichtenstein, Oliverio & Goldman, 1969), and this applies even to the highly MTX-sensitive Yoshida sarcoma, where uptake is inhibited by high concentrations of folic acid in the medium (Divekar, Vaidya & Braganca, 1967).

The normal renal tubular re-absorption of folic acid is blocked by MTX, and this can lead to a loss of folate from the body (Goresky, Watanabe & Johns, 1963; Hoffbrand & Fry, 1972). Because renal elimination of MTX is so important, drug toxicity is greatly increased in patients with renal failure (Burchenal *et al.*, 1951; Condit, Shnider & Owens, 1962; Condit, Chanes & Joel, 1969; Ojima *et al.*, 1970). It has even been suggested that elimination of MTX could be useful as a sensitive index of renal function (Zubrod, 1971). Besides renal failure, toxicity of MTX can be increased by bone marrow insufficiency, or by the concomitant administration of testosterone (Condit, 1960a). In human patients, there is a wide variation in the amount of MTX which can be tolerated, and the only important variable of value in predicting toxicity is said to be age (Hansen *et al.*, 1971). It is especially noteworthy that an attempt at suicide by overdose of MTX produced no symptoms of toxicity, but there was a tenfold increase in leukocyte thymidine kinase levels (Wilmanns, 1971).

It is probable that membrane transport of MTX, and of other folates, plays a part in their action by limiting access (Goldman, 1971). Flux across the membranes is potentially a two-way process, as indicated by the displacement studies, although it is usually one-way because of the tight binding between MTX and DHFR. Although MTX can penetrate normal skin (Newbold & Stoughton, 1972), its lack of effect when applied topically to psoriatics is well known. The work quoted here, and in a previous publication (Newbold, 1973), indicates that a central effect of MTX is needed in psoriatics, as previously suggested (Comaish, 1969). It follows, therefore, that the risk of liver damage cannot be separated from the therapeutic action of MTX.

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Case Reports

Cheyletiella sp. in the personal environment

WITH NOTES ON THE DIFFERENCES BETWEEN *C. PARASITIVORAX* MÈGNIN AND *C. YASGURI* SMILEY

M.HEWITT AND S.M.TURK

Royal Cornwall Hospital, Truro, Cornwall

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SUMMARY

Cheyletiella sp., with other mites, was isolated from the underclothes shakings of a woman with a papular rash and chronic urticaria. She had no contact with pets. Previously, *Cheyletiella* has been recognized only as an infestation derived from animals. The possible significance of this finding is discussed. The minute morphological differences between *C. parasitivorax* and *C. yasguri* are described.

In studies of the microbiology of the apparently healthy skin, very little attention has been paid to mites as members of the microfauna. Nor is very much known about the mites which are regularly or occasionally found in man's immediate environment under different climatic conditions. Our earlier experience (Hewitt *et al.*, 1973) suggests that many species may be found on the skin and in the clothes. Only further critical investigations, with reliable identification of the mites, will establish which species are capable of causing symptoms in man, and by what mechanisms.

CASE REPORT

M.W., a housewife aged 43 years, of neat and clean appearance, with a part-time canteen job, was first seen 6 years ago complaining of severe ano-genital pruritus; clinically, there was perianal erythema and moistness. The symptoms continued and 2 years later she was seen again with severe irritation of the scalp, and a papular rash on the hair margins resembling acne varioliformis. Later she developed a recurrent papular rash on the scalp and hair margins, and general urticaria. In July 1972, she reported with severe general pruritus, and increased skin sensitivity was noted; there was also a papular rash on the upper limbs and trunk. No causative factors were identified; her home was clean, tidy and well-kept. Shakings from clothes in contact with the rash were examined at the Public Health Laboratory, Truro, by the method described by Hewitt *et al.* (1973) with the following results (Table 1).

TABLE 1. Laboratory findings

Date	Source	Acari
21 August 1972	Clothes shakings	*Cheyletiella* sp. ♂ (too damaged for specific determination); *Dermatophagoides pteronyssinus* Trt. ♀
21 August 1972	Skin brushings	Negative
23 November 1972	Clothes shakings	*D. pteronyssinus* ♀
30 November 1972	Clothes shakings	*D. pteronyssinus*, nymphs; *Glycyphagus* sp.
27 December 1972	Clothes shakings	*D. pteronyssinus*, two nymphs
27 May 1973	Nightdress shakings	*D. pteronyssinus* ♂ ♀ and nymph; *Tyrophagus palmarum* Oud. ♂

NOTES ON *CHEYLETIELLA*

At present, acarologists recognize five species of *Cheyletiella* in the world (Ewing, Mosier & Foxx, 1967), of which apparently only one (*C. parasitivorax*) had been implicated in human skin rashes until Smiley (1965) described *C. yasguri* from dogs in the U.S.A. Since then, all specimens taken from dogs

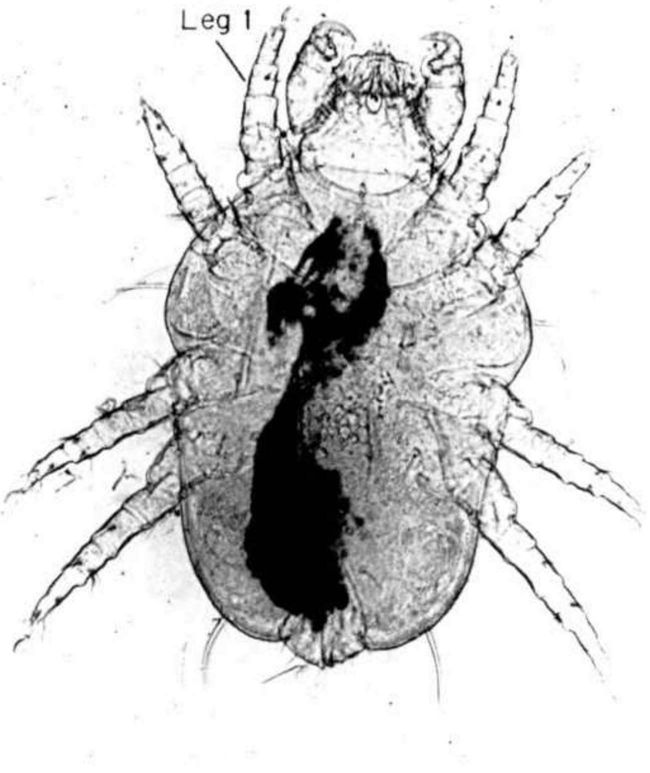

FIGURE 1. *C. yasguri* ♀, dorsum.

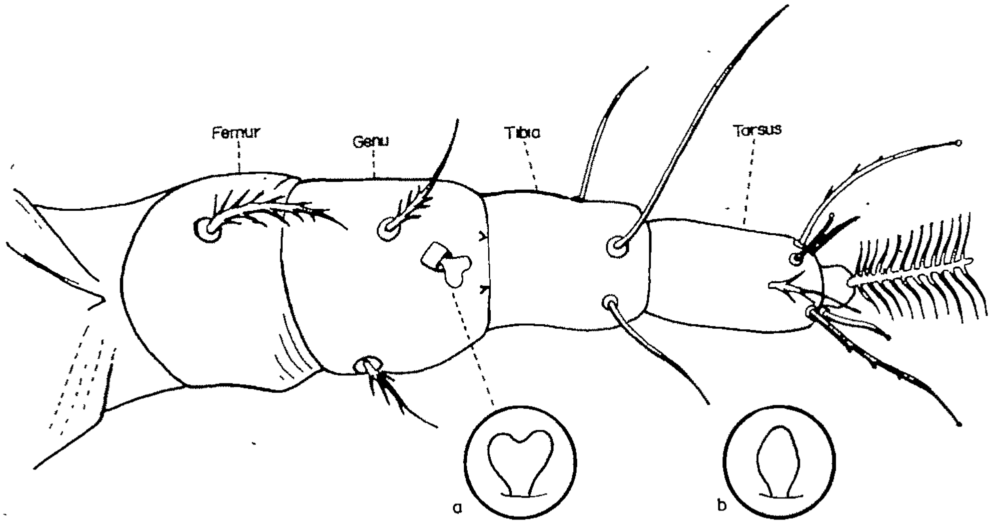

FIGURE 2. *C. yasguri* ♀, leg 1. (a) Sensory organ of genu. (b) Sensory organ of *C. parasitivorax*.

and nearly all those from cats and humans have been found to be *C. yasguri*, whilst those taken from rabbits have been determined as *C. parasitivorax*. Apart from the fact that the dorsal setae in *C. yasguri* are longer and wider than in *C. parasitivorax*, the only difference appears to be in the shape of the sensory organ on the genu of the first pair of legs (Figs. 1 and 2). According to Muller & Kirk (1969), cases of canine dermatitis due to *C. yasguri* are increasing. *C. parasitivorax* was first described by Mègnin (1878) as a free-living mite in the fur of rabbits, preying on fur mites and, for many years, *Cheyletiella* spp. were believed to be predators, like most other members of the family Cheyletidae. When the first cases of cheyletoid dermatitis in animals and humans were reported, these were thought to be due to a population explosion of *Cheyletiella*, accompanied by exhaustion of the supply of smaller mites such as ear mites (*Otodectes*), fur mites (*Listrophorus*) and follicle mites (*Demodex*) on which they were assumed to feed (Cooper, 1946; Ford, 1944; Hirst, 1917; Kuscher, 1940). Koffman & Viriden (1949) suggested that these mites are in a transitional stage between predator and parasite. More recently, the role of *Cheyletiella* as obligatory parasites has been postulated by Beesley (1963) and Moxham, Goldfinch & Heath (1968), who believed that the theory that they are predators can be traced to Mègnin's original and, in their view, unconvincing account of the mite's behaviour when placed in a glass jar with rabbit fur mites (*Listrophorus gibbus* Pagenstecher).

DISCUSSION

Human skin eruptions from contact with animals infested with *Cheyletiella* have been widely reported by Davies (1938, 1941), Hewitt, Walton & Waterhouse (1971) in the U.K., Bakkers & Fain (1972) in Belgium, Olsen & Roth (1947), Bjarke, Hellgren & Orstadius (1973) in Scandinavia, Moxham *et al.* (1968) in New Zealand, Rack (1971) in Germany, Taylor (1969) in Australia and Ewing *et al.* (1967) in America. *Cheyletiella* has not, however, previously been reported on humans in the absence of animal contact, although Bronswijk, Jansen & Ophof (1974) have been investigating a house infestation in Holland. In the patient M.W., three other species of Acari were repeatedly found in her clothes shakings during a 12-month period and the significance of their presence is not yet fully understood. *D. pteronyssinus*, however, is already known to be the cause of various allergies (Bronswijk & Sinha,

1971) and similar cases have been recorded in the literature involving *Glycyphagus* spp. and *Tyrophagus* spp. (Rook, Ebling & Wilkinson, 1968). It seems possible that prolonged exposure to these mites may account for the increasing skin sensitivity observed in this case. Whether the role of *Cheyletiella* was in this instance that of predator or parasite cannot at this stage be judged. The further significance of this case may lie exactly in this; if this is a predator and a facultative parasite as some believe, then *Cheyletiella*-like rashes can occur in patients sensitized to other allergy-producing Acari, vagrant specimens of *Cheyletiella* reaching the patient whilst in pursuit of their normal prey.

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Carcinoma of the larynx in two patients with keratoacanthoma

R.S.CHAPMAN AND O.A.FINN

Department of Dermatology, Falkirk Royal Infirmary, Stirlingshire, Scotland

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SUMMARY

Two cases of poorly differentiated squamous cell carcinoma of the larynx are described, one occurring in a patient with a solitary keratoacanthoma, the other in a patient with multiple keratoacanthomas. Investigation and treatment in the conventional manner are strongly advised. Any temptation to delay, based on the rare entity of benign eruptive keratoacanthoma, should be resisted.

Keratoacanthoma or molluscum sebaceum is now well known to dermatologists and to surgeons as a benign self-healing tumour. Numerous reports in the early 1950s defined the clinical and histological features, emphasizing the benign nature and the ultimate spontaneous regression of the lesion (Rook & Whimster, 1950; Whittle & Lyell, 1952; Beare, 1953; Calnan & Haber, 1955).

The occasional case of multiple keratoacanthoma has been reported (Borrie, 1958), where more than one keratoacanthoma occurs in the same individual, each tumour having an appearance similar to that of the solitary lesion. Much rarer is the so-called eruptive type (Baer & Koph, 1962) where hundreds of tumours, from a pin-head upwards in size, develop on the skin. Several cases of this type have had laryngeal symptoms (Witten & Zak, 1952; Rossman, Freeman & Knox, 1964; Jolly & Carpenter, 1966; Winkelmann & Brown, 1968). It is generally accepted that the laryngeal involvement is a part of the syndrome in this rare condition of eruptive keratoacanthomas, and that any accompanying laryngeal tumour is benign, although requiring biopsy and continuing observation.

We wish to report two cases, one of a solitary keratoacanthoma, the other of multiple keratoacanthomas, in whom histologically proven squamous carcinoma of the larynx developed before or subsequent to the skin tumours.

CASE REPORTS

Case 1

W.McR., a mentally retarded male aged 72, presented in June 1970 with a 3-month history of a rapidly growing warty lesion on the chin. A smooth rounded tumour, 1·75 cm in diameter, with a central keratin plug, was noted on the chin, extending to involve the vermilion of the lower lip. A clinical diagnosis of keratoacanthoma was made, and, in consultation with a surgeon, it was decided to await spontaneous healing. In July 1970, the tumour was 2·5 cm in diameter with a large central mass of keratin. By October 1970, this keratinous plug had detached, leaving a depression with craggy irregular margins. No enlargement of neck lymph glands was noted at any time. The patient was kept under review for the following 18 months without recurrence and with cosmetic improvement in the scar.

Towards the end of 1972, persistent sore throat and hoarseness led to referral to the ENT Department, where laryngoscopy showed considerable swelling of the mucosa over the left arytenoid, which was replaced by friable boggy tissue. Biopsy showed a poorly differentiated keratinizing squamous cell carcinoma. The laryngeal epithelium showed dysplastic changes. Serial sectioning revealed no 'shoulder' to suggest keratoacanthoma. A lymphocytic infiltrate surrounded the masses of tumour cells. The patient has since received radical radiotherapy to the larynx.

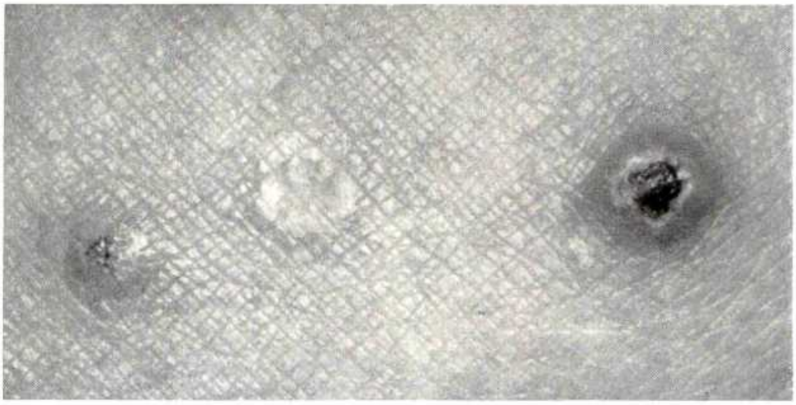

FIGURE 1. Left elbow (Case 2) with three keratoacanthomas at different stages of development.

Case 2

J.C., a housewife aged 49, was seen originally in surgical outpatients in June 1970 with a warty lesion, 1 cm in diameter, on the lower forehead. The surgeon accepted the histological report of keratoacanthoma, but review of the section has failed to confirm this diagnosis, the features being those of an actinic keratosis: alternating columns of hyperkeratosis and parakeratosis in the stratum corneum, some follicular plugging and dysplastic changes in the basal layer.

In December 1970, she was referred to the ENT Department with a complaint of occasional hoarseness and throat pain dating from June 1970. At direct laryngoscopy, thickened mucosa was observed in the left pyriform fossa, post-cricoid area and ary-epiglottic fold extending to the tongue. Biopsy from the left pyriform fossa showed a poorly differentiated keratinizing squamous cell carcinoma. A course of radical supervoltage radiotherapy was given to the larynx and to the left mid-cervical nodes, which were clinically involved. Continuing observation has failed to show any evidence of relapse.

In August 1972, she noted the development of nodules on the dorsum of the right hand, the right forearm and the right calf. Clinically, these were typical keratoacanthomas. Similar tumours have continued to appear, particularly on the helices of the ears, on the cheeks and on the forearms (Fig. 1). One tumour excised from the right arm showed the typical histological features of an involuting keratoacanthoma; the shoulder of normal epithelium enclosed a cavity lined by acanthotic epithelium containing the remains of a keratin plug, and with a chronic lymphocytic infiltrate in the underlying dermis. She has now developed over a dozen typical tumours, fresh keratoacanthomas appearing as others resolve spontaneously.

DISCUSSION

Symptoms and signs related to the oropharynx, apart from the case of Reager (1968), have been described only in eruptive keratoacanthoma. Witten & Zak (1952) reported a patient with eruptive keratoacanthoma and attributed his hoarseness to prolapse of the left ventricular fold over the left vocal cord, perhaps by a tumour which was not visualized. Reddening of the cords, one of which had a small defect, and white streaks suggestive of leukoplakia on the buccal mucosa were also noted. The patient of Rossman *et al.* (1964) had several nodules removed from the vocal cords, all originally described as well-differentiated squamous cell carcinoma, although the authors considered that the acanthosis and dyskeratosis seen in the biopsies together with the lack of progression and the time of onset favoured an interpretation of keratoacanthoma of the larynx. One of three patients described by Winkelmann & Brown (1968) had previously received radiotherapy for a tumour of the larynx thought to be squamous cell carcinoma. White scarred areas were seen on both vocal cords 3 years later. This patient had microstomia as a consequence of his disease, as had a second patient who had many white-topped papules over the epiglottis and the antero-ventral surface of the tongue. An earlier laryngeal biopsy in this patient had shown the features of an infiltrating squamous cell tumour with some foci of bizarre cells, interpreted originally as grade 1 squamous cell epithelioma but considered by the authors to be comparable with the skin lesions of keratoacanthoma. Unfortunately, there was no mention of laryngeal examination at autopsy. Their third patient had no oral or laryngeal symptoms. The patient described by Grzybowski (1950) had no symptoms referable to the larynx, although he showed many warty papules on the palate, with white macules and coalescent papules over the buccal mucosa. The patient of Jolly & Carpenter (1966) had hoarseness, as well as nodules on the buccal mucosa. Reager (1968), under the diagnosis of multiple keratoacanthomas of Ferguson-Smith, reported a man who showed massive papillomatous lesions on the epiglottis and false cords at laryngoscopy. Several laryngeal biopsies had shown irregular acanthosis with some individual cell keratinization and horn pearl formation which resembled the picture seen in the skin lesions. In view of lack of progression of the laryngeal signs, laryngoscopy and biopsy at intervals was recommended.

Each of our two cases described above had a poorly differentiated squamous cell carcinoma of the larynx. We do not claim any causal relationship between the presence of solitary or multiple keratoacanthomas and carcinoma of the larynx. This is reinforced by a review of the last thirty patients with squamous cell carcinoma of the larynx seen at our hospital having had no skin tumours of keratoacanthoma type, either preceding or following symptoms of the laryngeal cancer. Conversely, none of our past twenty histologically proven cases of keratoacanthoma has developed any laryngeal symptoms, although one had had an adenocarcinoma of the colon excised 30 months prior to the appearance of the keratoacanthoma.

Although eruptive keratoacanthoma may be accompanied by apparently benign laryngeal lesions, the clinician should not be misled into arguing or postulating that laryngeal involvement in the case of single or multiple keratoacanthoma can be treated expectantly. We would recommend detailed investigation and management along accepted surgical lines for any patient with solitary or multiple keratoacanthoma who develops laryngeal symptoms.

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Keratoacanthoma in a smallpox vaccination site

S.HAIDER

Department of Infectious Diseases, Royal Free Hospital, Coppetts Wood Hospital,
Muswell Hill, London N10 1JN

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SUMMARY

Tumours arising at the site of smallpox vaccination are rare. A case of keratoacanthoma developing within 2 months at the site of vaccination is described and a likely role of vaccinia virus in the causation of such a tumour is discussed.

Complications of varying type and severity may follow smallpox vaccination. Except for encephalitis, these are not officially notifiable diseases, and thus available statistics on their incidence are often unreliable; however, a primary tumour developing in the site of vaccination is certainly rare. Lane *et al.* (1970) in 1,648,000 vaccinations against smallpox described one patient who developed melanoma. We describe here a case of keratoacanthoma developing within 2 months at the site of a successful primary vaccination.

CASE REPORT

A 55-year-old woman, 3 weeks after her primary vaccination on the left deltoid region, developed a nodule under the scab of her vaccination. When seen at this hospital 8 weeks later the tumour was a solitary dome-shaped growth, about 1 cm in diameter, with a central horny crust (Fig. 1). There were

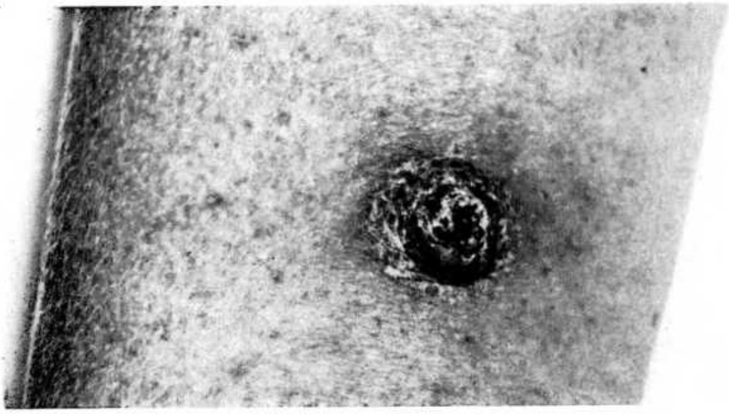

FIGURE 1. Keratoacanthoma at the site of vaccination.

no signs of inflammation or ulceration in or around the tumour and no associated axillary lymphadenopathy. The patient had no other constitutional disturbance. Biopsy of the tumour showed this to be a keratoacanthoma. Electron microscopy and immunofluorescence studies for the vaccinia virus were negative, and no virus was isolated.

DISCUSSION

Vaccinia gangrenosa and satellite lesions are fairly well recognized early complications at the site of vaccination. There is also growing evidence from clinical studies in man that immunization against smallpox has been followed by the development of tumours, such as basal cell carcinoma, melanoma (Marmelzat, 1968) and squamous cell carcinoma (Rea, 1956), in the vaccination scar. Goncalves (1966) described one case of pigmented basal cell carcinoma, and while reviewing the subject of malignancies arising in vaccination scars mentioned seven more cases—two squamous and five basal cell carcinomas. These tumours have usually developed many years after the vaccination and may perhaps be no more than tumours developing in scar tissue, the initial cause of which may have little relevance.

Viruses of the pox group are known to cause self-limiting neoplasia (fibromas, myxomas) in rabbits. Borrel (1903) and Bosc (1903), working separately, pointed out that vaccinia virus not only destroys the cells but also exerts a proliferative effect on them, although malignant transformation in such proliferative changes is rare (Goodpasture, 1957). F. Duran-Raynals (1957) suggested a carcinogenic or co-carcinogenic potential of the vaccinia virus, and his work was confirmed by M. L. Duran-Raynals in 1962. Koziorowska, Wlodarski & Mazurowa (1971) also showed that *in vitro* infection with vaccinia virus of mouse embryo cells leads to their neoplastic transformation.

In our patient, in whom this tumour developed so soon (within 2 months) at the site of vaccination, it is tempting to speculate that the mechanism of tumour induction was in some way related to the proliferative effect of the vaccinia virus, although no virus was isolated from the lesion.

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Erythroderma in a child with coeliac disease

HAROLD WOOLFSON, ALEXANDER McQUEEN AND
MARY STEPHEN

Departments of Dermatology, Stobhill Hospital, Glasgow, and University of Glasgow

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SUMMARY

Erythroderma is described in a 2-year-old child with coeliac disease, who died of fungal pneumonia. The skin eruption commenced the day after a gluten-free diet was instituted and proved intractable to treatment, whereas the signs of malabsorption were swiftly controlled.

We could establish no connection between the cutaneous and intestinal disorders.

Pruritic eczema of seborrhoeic or psoriasiform pattern with a tendency to generalize and desquamate has been reported as a cutaneous manifestation of coeliac disease (Wells, 1962; Friedman & Hare, 1965). Though a considerable minority of adults are affected (Cooke, Peeney & Hawkins, 1953; Badenoch, 1960), coeliacc hildren are relatively exempt (French, Hawkins & Smith, 1957). Most often the skin signs have antedated the overt effects of malabsorption and have improved when a gluten-free diet was instituted.

CASE REPORT

A boy aged 2 years and 4 months, sixth child of a family of seven, was admitted to hospital on 23 July 1972 for investigation of diarrhoea intermittently present for 1 year. There was no history of previous skin disorder nor was there a family history of skin or of coeliac disease. He was a friendly happy child with a 'dry' skin, protuberant abdomen, thin buttocks and a liver edge palpable 3 cm below the right costal margin. His height was 78 cm (below the third percentile of height for his age) and his weight was 14 kg (at the tenth percentile for his age).

No infective cause was found for the clay-coloured diarrhoea, which was interrupted by occasional episodes of constipation and abdominal distension. Pediculosis and impetigo of the left postauricular region of the scalp were noted, and coagulase positive *Staphylococcus aureus* phage type 71 and group A haemolytic streptococci were cultured. Throat and nasal swabs showed normal flora, and no virus was grown on tissue culture. The impetigo responded well to local antibiotic treatment.

Coeliac disease was diagnosed on the result of jejunal biopsy, on the presence of steatorrhoea and on malabsorption of xylose and possibly iron. Two features not in keeping with coeliac disease were the cheerful temperament and the hepatomegaly. The diarrhoea was relieved and the blood picture improved within weeks when a gluten-free diet with iron and vitamin supplements was commenced.

The day following the start of this regime, a pustular folliculitis was noted, with scaling and crusting of the right post-auricular region, which spread rapidly to the neck and side of the face. Coagulase-positive *Staph. aureus* was cultured, and antibiotic treatment was applied locally. Within 10 days the boy had developed a severe and widespread erythematous reaction with large adherent opaque scales and yellow crusting of the scalp and eyebrows (Fig. 1). Initially the eruption showed a 'seborrhoeic' distribution, but after the development of a crop of clear non-umbilicated vesicles the erythroderma became exudative and the scaling universal. The vesicles did not recur, nor was there evidence of a viral or bacterial origin. Viral studies and blood cultures were negative. The appearance of the vesicles did not suggest psoriatic pustulation. By now the child was irritable, febrile and toxic. There was a mild generalized non-suppurative lymphadenopathy. Numerous local applications—bland, antiseptic, antibiotic, anti-candidal (though skin scrapings were sterile on culture) and steroid—were tried without sustained benefit. In addition, as the patient developed frequent upper respiratory tract infections, systemic antibiotics were given. Oral diiodohydroxyquinoline was ineffective. The illness continued with unabated severity, and prednisolone at an initial daily dose of 45 mg was commenced. His general state improved, the pruritic erythroderma lessened, the scaling was converted from large to fine psoriasiform type. The prednisolone was gradually reduced and was discontinued after 1 month. In a few days, both the systemic and cutaneous illness recurred with 'steroid rebound' vigour. Tetracosactide zinc phosphate complex was started. Again this at first gave good but unfortunately only very temporary control. There was loss of scalp hair and finger nails. The frequency of injections was reduced but it was never possible for the remainder of his life to discontinue this treatment.

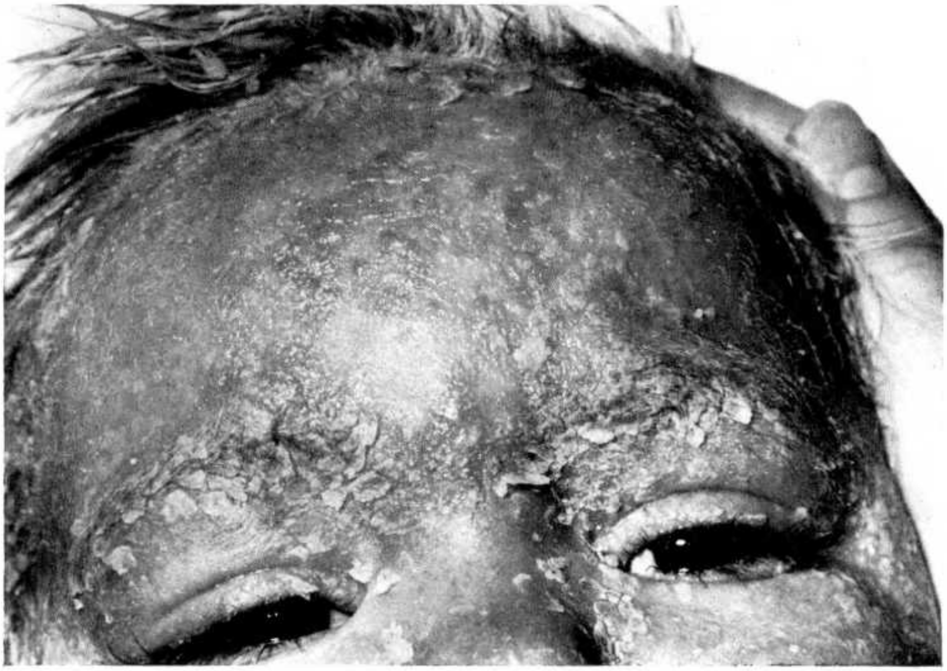

FIGURE 1. Early eruption on scalp and forehead.

The child had rapidly developed marked Cushingoid features, so much so that he was considered to be unusually sensitive to steroid effects. There was some retention of fluid, but diuretics controlled this and though the liver continued to enlarge there was no ascites.

After 3 months stormy illness the patient developed clinical and radiological signs of acute bronchopneumonia. In spite of intensive treatment he died within 3 days.

Investigations

Urinary xylose excretion was equal to 8% of a 5 g oral dose. Daily fat excretion (5-day collection) was 9 g. Jejunal biopsy: sub-total villous atrophy. Haematology: Hb 9·5 g/100 ml, PCV 33%, MCHC 28%, WBC 6000/mm^3. Differential count: neutrophils 47%, lymphocytes 35%, eosinophils 10%, monocytes 8%. ESR (Westergren) 19 mm/1 h. Serum iron 45 μg/100 ml, TIBC 345 μg/100 ml, saturation 13%. Tibial marrow was normal but no stainable iron was present.

Skin biopsy (*right leg*). Histological studies revealed a psoriasiform tissue reaction in the epidermis, which included in places small collections of leukocytes under parakeratotic keratin resembling early Munro type micro-abscesses. In other areas there was marked spongiosis amounting to microvesicle formation in places. A few non-specific inflammatory cells were seen scattered through the epidermis, and the papillary dermis showed considerable oedema. The papillary capillaries were widely dilated, but lacked the rigid appearance characteristic of psoriasis, and many 'squirting' papillae were seen. The dermal infiltrate consisted of a mixture of polymorphonuclear leukocytes, lymphocytes, histiocytes and occasional eosinophils. No fungi were seen on examination of appropriately stained sections.

These appearances were interpreted as those of a severe dermatitic reaction, and as such not diagnostic, but certainly consistent histologically with those described in seborrhoeic dermatitis (Pinkus & Mehregan, 1969).

All other investigations were normal; these included sweat electrolytes, blood biochemistry, immunological studies for food antibodies, serum immunoglobulin levels, nitro-blue Tetrazolinium dye test, and chromosome analysis. Ante-natally the mother had been well and had no serological evidence of syphilis.

Post mortem findings

Examination was performed 8 h after death. The only abnormalities recorded on naked eye examination were bronchopneumonia and hepatomegaly (weight 770 g, i.e. approximately 1·5 times the expected weight). The cut surface of the liver appeared normal. There were a few enlarged lymph nodes in the axillae, groins, mesentery and hila of the lungs. Histological examination confirmed the diagnosis of bronchopneumonia, but the striking finding was the presence of fungal hyphae in the parenchyma of the lung and within its blood vessels. These hyphae were also found within the blood vessels of the brain and in the meningeal vessels, and indicate opportunistic fungal infection which had become disseminated via the vascular system. In spite of the large size of the liver, only minimal fatty change was found. Many hepatocytes, however, were swollen, and patchy cellular necrosis was evident. An increase in pericellular fibrous tissue was apparent, and no iron could be demonstrated using appropriate staining methods. An axillary lymph node showed the typical appearances of dermatopathic lymphadenitis, and the adrenal glands gave the expected picture of atrophy associated with corticosteroid administration.

No significant lesion was found in the spleen, kidney, myocardium or pituitary gland.

DISCUSSION

A primary systemic or cutaneous cause, whether ascertainable or not, has been said to underlie all cases of erythroderma (Rook, Wilkinson & Ebling, 1972). Neither during life nor at post mortem was a systemic cause found in this case. It does not seem likely that the coeliac disease could have been the prime cause, for only after it was treated and controlled did the erythroderma develop.

The positive findings at post mortem were the fungaemia and the hepatomegaly. Fungal pneumonia was the immediate cause of death and was not a cause of the erythroderma, which had been present for 3 months prior to this acute termination of the illness. It seems probable that the steroid therapy had suppressed the child's immune responses and that the antibiotic treatment had provided a clear field for the invasion and dissemination of the fungus. Though recognized morphologically as fungus, we were not able to culture the pathogen from post mortem material.

The enlarged liver, found at post mortem to be histologically normal, had been noted before the erythroderma appeared. It became marked only late in the illness and may have been due in part to the androgen effect of steroid treatment. However, Abrahams, McCarthy & Sanders (1963), in their study of 101 cases of adult exfoliative dermatitis, found an enlarged liver present in twenty cases. In eleven of these, the aetiology of the exfoliation was undetermined. They concluded that hepatomegaly can be a non-specific finding in exfoliative dermatitis.

No external factors were found responsible for the severe dermatitic reaction. Clinical observation did not suggest an allergic or irritant contact pathogenesis. The staphylococcus isolated from the pustular folliculitis which heralded the generalized eruption might have had an initiating rôle but there was no bacteriological or histological evidence that it remained continuously operative for 3 months.

The skin histology showed features of both eczema and psoriasis. The 'dryness' of skin noted when the patient was being investigated solely for malabsorption and ascribed then to this cause (Wells, 1962) might instead have indicated atopy. There was, however, no family history of this or of psoriasis. Frost & Van Scott (1966) conveniently considered psoriasiform erythroderma among the ichthyosiform dermatoses, but recognized that it is atypical psoriasis. In two of their three cases the disorder was congenital, in the third it appeared at 2 years and became general at 4 years. All three had a positive family history and clinically and histologically were indistinguishable from generalized psoriasis. Earlier, Pascher & Wood (1956) reported two children suffering from erythrodermic psoriasis who showed typical histology.

In our patient the presentation suggested seborrhoeic dermatitis with secondary eczematization, itself a rarity at 2 years of age (Rook, Wilkinson & Ebling, 1972). Later in its course and perhaps influenced by steroids, the clinical picture was more compatible with psoriasiform erythroderma despite the presence of pruritus, lymphadenopathy and eosinophilia, three features more commonly found in non-psoriatic exfoliative conditions (Wilson, 1954). The histological appearances were consistent with both diagnoses, a combination usually found in older people (Ingram, 1954).

We could not identify any immunological deficiency state that might have contributed to the generalization of the eruption. Such deficiency states are commonly characterized by skin rashes (Jacobs & Miller, 1972). Only the presence of the coeliac disease provided the most intangible of evidence by its known association with lympho-reticular dysfunction (Crabbé, Douglas & Hobbs, 1970) and with selective IgA deficiency (Booth, 1970).

Recently, however, two families have been described with a genetically determined deficiency of phagocyte enhancement due to dysfunction of the fifth component of complement and with associated generalized seborrhoeic dermatitis, diarrhoea, recurrent infections and wasting (Jacobs & Miller, 1972). These are all features of Leiner's disease (Leiner, 1908), features shared to some extent by our patient. Unfortunately, we were unable to investigate for this defect. Failing detection of possible recondite factors such as this, the only cutaneous basis which was established for the erythroderma was an amalgam of two common disorders.

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Tinea barbae caused by *Trichophyton erinacei*

J.R.SIMPSON

Royal Devon and Exeter Hospital, Exeter, Devonshire

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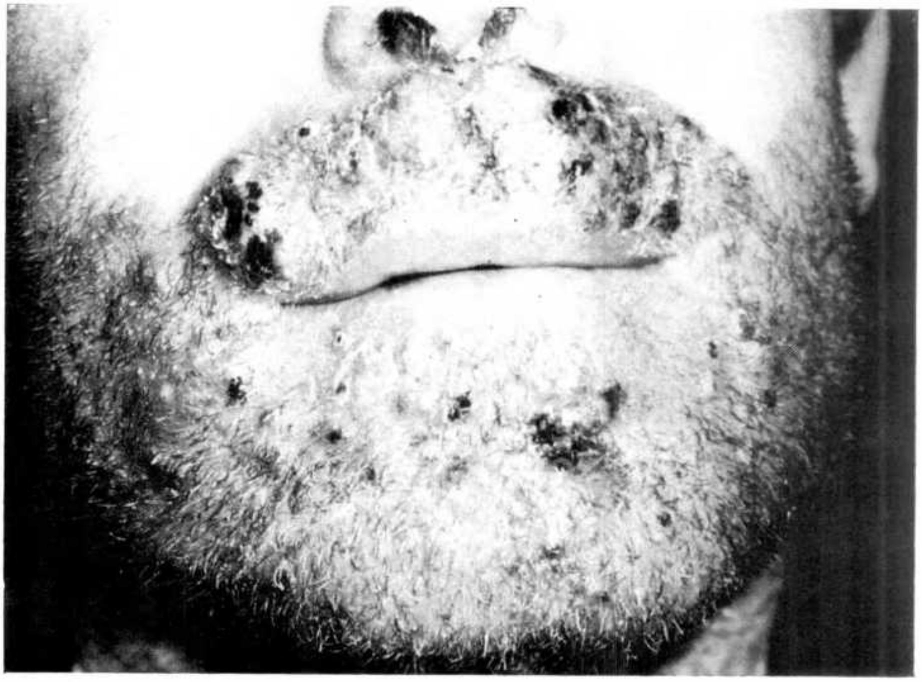
SUMMARY

A case of kerion barbae is reported due to *Trichophyton erinacei* with probable direct transfer to the patient's wife.

CASE HISTORY

A man aged 42 developed rough areas on his neck early in December 1972. He was treated with fluorinated corticosteroid creams, oral antibiotics and tranquillizers, but the lesions spread to the face and towards the end of February became acutely inflamed. On 5 March 1973, he presented with a typical kerion barbae which was rather more severe than usual and involved the whole of both lips, the chin and the adjacent parts of the cheeks and neck (Fig. 1). He was warned that the rash was contagious, and he was treated with griseofulvin 1 g daily orally and simple local hygienic measures.



As he operated a bulldozer and often worked or parked it in fields where cattle were grazing, it was assumed that he had contracted cattle ringworm. However, *Trichophyton erinacei*, the agent of hedgehog ringworm, was cultured from the infected hairs. When seen on 26 March 1973, he was found to be much better. He was accompanied to the clinic by his wife, who on 17 March 1973 had developed a pink annular lesion on the right side of the neck. *Trichophyton erinacei* was cultured from her lesion also, and it responded well to the inunction of Whitfield's ointment.

Further enquiry revealed that the husband had disturbed a hedgehog's nest with his bulldozer in October 1972 and had brought the two baby hedgehogs in it home for his daughter, but they had not survived for more than 3 weeks. The box they had been kept in, the straw, and the rags used to protect them, had all been destroyed, and the bodies had been buried in the garden. The patients kept two large dogs but had never seen them playing with live or dead hedgehogs.

DISCUSSION

Smith, Rush-Munro & McCarthy (1969) recorded 103 human cases of hedgehog ringworm in New Zealand, in which the lesions were on the face in eleven, on the neck in three, on the leg in thirty-three and on the scalp in one case. The morphology of the lesions is not described in detail but there were no beard infections and there was no specific mention of *Trichophyton erinacei* causing a kerion. Accounts of about twenty cases of *Trichophyton erinacei* infection in man in Britain have been published, but no case of tinea barbae has been recorded.

The New Zealand authors pointed out that the maximum incidence of human infection with *Trichophyton erinacei* in that country does not correspond with the warmer months of the year when hedgehogs are more active and not hibernating. They concluded that, as very few other animal species have been found harbouring this fungus and as *Trichophyton erinacei* appears incapable of prolonged survival or growth in soil (English, Smith & Ruch-Munro, 1964), most human infections result from indirect contact with hedgehogs via infected debris. English *et al.* (1962) suspected that dogs hunting hedgehogs could act as vectors.

In this family, the wife's infection appeared at least 3 months after all traces of the hedgehogs had been removed from the house, and the dogs did not hunt hedgehogs. It therefore seems likely that she contracted her infection directly or indirectly from her husband.

ACKNOWLEDGMENT

I would like to express my thanks to Dr Mary English for isolating and identifying the fungus and for much other help and advice.

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History of Dermatology

Dermatology and history in Wales (Cymru)*

GEOFFREY HODGSON

University Hospital of Wales, Cardiff

SUMMARY

The development of dermatology in Wales (Cymru) is traced against a short history of the independent kingdom which became the Principality. Skin diseases were treated by the earliest Cymry, Celtic druids, Celto-Christian priests, Anglo-Saxon leeches and court physicians, and later by medieval university graduates and unskilled village herbalists and, when industrialization came, by 'truck doctors' and general practitioners. The early dermatologists are described. Traditional holy wells and folk medicine form connecting links.

The mountains in Wales have made its history and dermatology. Sheltering the Cymry, driven by invaders from Britain into the present confines of the Principality, they isolated them as a pastoral people until iron and coal in the eighteenth and nineteenth centuries initiated the modern changes. Even in 1840, the only towns of any size were Merthyr Tydfil with 22,000, Swansea with 15,000 and Cardiff with 6000 persons (Jones, 1972).

Peculiar to Wales are its triads (Trioedd). These are prose compositions with terse statements of three related facts. The bards used them to help people to remember. The oldest, which were historical, ethical, theological and legal, were collected by Caradoc of Llancarfan in the twelfth century in 'Historie of Cambria', translated by Humphrey Llwyd, from a text of Brut y Tywysogion. Some persist today as Welsh proverbs (Diarhebion Cymraeg), for example, 'three things that are difficult for a man—to cool the fire, dry the water and please the world'. A Glamorgan tribon, or verse form of a triad, runs:

'Tri pheth syn hynod poenus	(Three things which are remarkably painful,
Sef dafaden ar y wefus	Namely, a wart cancer (Dafaden wyllt) ('wild sheep') on the lip,
Cornwyd mawr o dan yr ên	An abscess under the chin,
A gwên yr eneth glwyfus'.	And a smile of a wounded girl.)

In the background of Welsh dermatology there are always the mountains with their streams running into holy wells. There are also the herbs of their valleys, whose healing mysteries were known to Celtic druids and Celto-Christian priests, to be recorded later in medieval manuscripts and to mature ultimately as modern drugs, or to persist as superstitions or as country treatments for warts even today.

PREHISTORIC TIMES

Presumably dermatology in Stone Age tribes (Palaeolithic 12,000–8000 B.C. and Megalithic 6500

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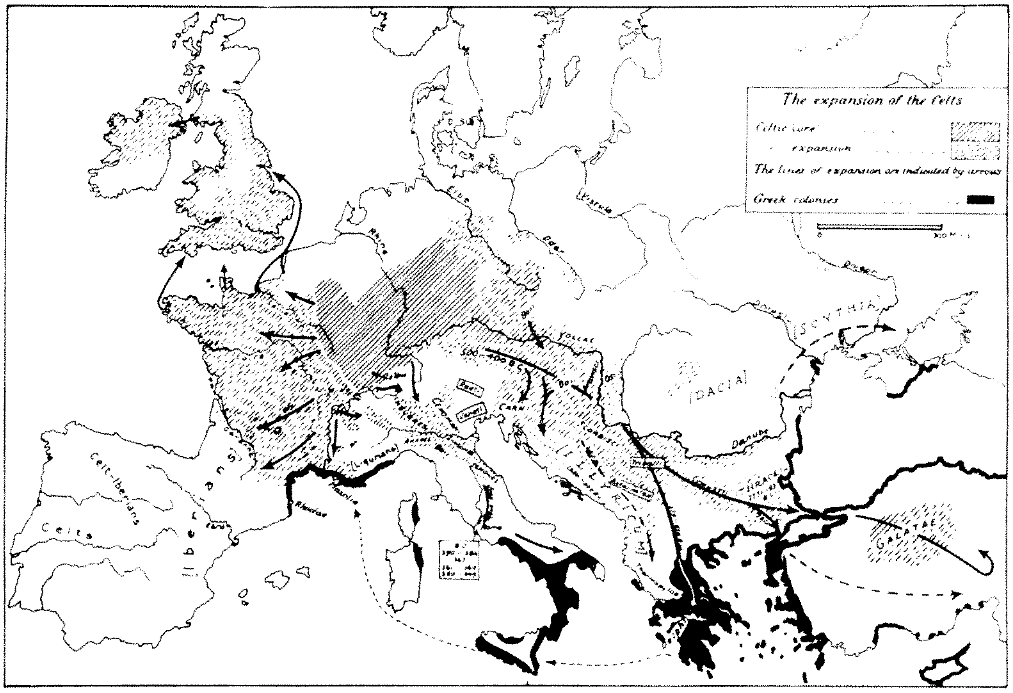
* Presidential address to the British Association of Dermatologists, July, 1973.

B.C.) consisted of the treatment of minor wounds, and the removal of stones and parasites by tribal mothers moistening, rubbing or squeezing the skin (Cule, 1970). This is mentioned in a medieval Welsh leech book, 'What is the simplest remedy?', followed by the answer, 'Scratch thy hand until it smart and then spit on it' (Jones, 1959).

EARLY CYMRY AND CELTIC PEOPLE

Some of the ancient Cymry came from Iberia (modern Spain and Portugal) to which they had migrated from Hindustan and the Middle East. Later came others such as the Beaker folk (2000–1800 B.C.) with bronze weapons and distinctive pottery (Chadwick, 1971). Medicine (Meddyginiaeth) was one of the rural arts practised by the Cymry. The bardic-priests were the teachers of knowledge (Gwybodaeth), which included the healing herbs.



EXPANSION OF THE CELTS (3rd to 1st Century B.C.).

FIGURE 1. Expansion of Celts. Reproduced by permission of Faber & Faber Limited from *An Historical Atlas of Wales.*

In the mountains lived the Welsh goat of ancient Cymru (Burroughs Wellcome, 1903), mentioned by Shakespeare in Henry V ('not for Cadwaladr and all his goats'). Then it was used for food, and its blood for medicine, now it is the parade mascot of the Welsh Regiment.

There followed, in 500 B.C., a new people. These were the Celts (Keltoi or Galli) from the region of the Upper Danube, now Austria and Czechoslovakia. They spread over Europe to Italy, Belgium, France, Spain, and they even formed a state in Turkey, as St Paul's Epistle to the Galatians reminds us (Fig. 1). Their influence declined in the second century B.C. before the conquering Romans. They were fair-skinned, blond, with flowing moustaches and swept-back hair, which may explain why cancer of the skin is said to be most common in those of Celtic ancestry (Urbach, Davies & Forbes, 1966). The Goidel Celts (Gaels) went to Ireland and later to Scotland; they spoke Gaelic. The Brythons, in Wales, spoke the first written language of Britain, which is the parent language of the Welsh of today and was spoken also in the north of Cornwall, and which is the concern of many Welsh people today.

The bards were, in the tenth century, at the Court of Hywel Dda ('Howell the Good' (A.D. 900–950) (Fig. 2)), divided into Chief bard (Pencerdd), bards of the royal Household (Bardd Teulu) and minstrels (Cerddorion). Later and in medieval times there were also bards on circuit (Cwrs) to the gentry and itinerant begging bards (clerwyr) (Archdruid Cynan, 1973).

FIGURE 2. St David, 'Dewi Sant'. Reproduced by permission of the Wellcome Institute of the History of Medicine from *Ancient Cymric Medicine.*

Gorsedd of Bards

The original ovate (green robes), bards, musicians, and literati (blue), and druidic (white) orders, today compose the Gorsedd of the bards, who now proclaim the annual National Eisteddfod. This is a competitive congress of bards, the earliest recorded gathering of which was in Cardigan in 1176, under the patronage of Lord Rhys.

In Celtic times the druids, being responsible for healing, would have treated skin disease.

Their white dress signified holiness and purity, they carried out secret and religious rites, and they practised mysticism. They were next in importance to the King. There were three types of female druids, according to Irish sources (Moloney, 1919): the sisterhood with special powers of healing and divination, married women who lived a short time with their own husbands and then with the druids, and a menial class. Their mysterious rites of healing together with human sacrifices and with prophecies were performed in sacred groves.

They used applications of sacred herbs, such as the 'All Heal' (Pren Awyn) mistletoe, which had to be cut with special ritual by a golden sickle on the sixth day after the new moon in winter (Burroughs Wellcome, 1903). The sacred wheat was gathered in autumn, the oak in summer and the shamrock (the Irish emblem) in spring. Disorders were also treated by drinking from holy wells, dedicated to the Celtic healer, God Sirona, the source of sunlight and warmth (Wellcome Historical Medical Museum, 1928), and probably also to Mercury, Apollo and Jupiter. Treatment was also given at the temple of Nudd, or Noden, on the river Severn at Lydney (Cule, 1971).

ROMAN TIMES

It was nearly a century after Julius Caesar landed in 55 B.C. before most of Britain was conquered by Claudius (A.D. 43), with the defeat of Caractacus. But the Welsh tribes (the Silures of Monmouth and Glamorgan in the south, and the Ordovices of Powys in the north) withdrew into the mountains, and, nourished by the Druid stronghold in the island of Anglesey (Dodd, 1972), put up fierce resistance, until subdued in A.D. 74–78 by Agricola, who savagely destroyed the Druids' groves. Their exhortative resistance was, however, later carried on by bards (Archdruid Cynan, 1973). Conquered Wales was contained by legionary fortresses at Isca (Caerleon) (A.D. 90) in Monmouth, and at Chester (A.D. 102). When in 406 A.D. the Roman Emperor Constantine removed his troops, he left Wales and its dermatology much as they had been before the Romans came, except perhaps for some military practices based on Greek medicine.

THE EARLY WELSH KINGDOMS AND ANGLO-SAXON BRITAIN

During the Dark Ages, Wales was for some 200 years after Roman times sporadically invaded by Irish tribes who brought Christianity, then by the Saxons, the Norsemen and Danes, and finally by the Normans in the twelfth century. The Welsh fought also among themselves, between princes and between north and south, until union gradually came during the ninth and tenth centuries during the struggles for supremacy. King Gruffydd ap Llewellyn defeated the English king Harold, at Welshpool (A.D. 1039) and finally created the independent United Welsh kingdom. During these centuries several important events occurred in Wales. The Dragon of the great Pentragonship (the Welsh dragon of today's Welsh flag, from the standard of King Cadwaladr, the last Welsh king of Britain) had been recognized *circa* A.D. 420, when the Romans left. St David, 'Dewi Sant' (A.D. 462) (Fig. 2), the patron saint of Wales, founded a Celtic church and finally destroyed pagan druidism. The leek, tradition says, was worn by Welsh soldiers on St David's day A.D. 640 to distinguish them from Saxons dressed as Welshmen (Burroughs Wellcome, 1903). Later the Saxons built Offa's dyke, isolating Wales from Mercia (A.D. 700), and the great Welsh king Hywel Dda (Fig. 3) codified the Cymric laws (A.D. 900–950), which were later to be replaced by English common law.

Little is known of any dermatology in the early troubled years. Three groups of persons probably practised dermatology. First were the Celtic monasteries and clergy, until ordinances of the Popes in the twelfth and thirteenth centuries finally forbade the practice of medicine. They used herbs from cultivated gardens, cast out devils with insignia and continued to use the holy wells (ffynon). Among the holy wells of Wales there are some 437 named after Celtic Christian saints (Jones, 1954), and of the many others some had adjectival names indicating medicinal use. Outside the monasteries the leech doctors (Laece; the Anglo-Saxon word) did the healing 'dermatology'. They diagnosed 'Elf shot' (Grattan & Singer, 1956) by the magic puncture and denting of the skin, and gave treatment by blood letting, cauterization and talismans inscribed with magic words. Tumours, for instance, were exorcised by placing upon the man iris leaf on which was written, 'In nomine Patris et Filii et Spiritus sancti, Amen' (Jones, 1959). The dermatologist at the King's court was the Court Physician. King Hywel Dda (A.D. 900–950) had granted him, in precedence, the twelfth place at court with special privileges equal to those of the chief bard (Pencerdd). The Court Physician treated the royal household free, but he could charge a fee of twelve legal pence for 'a red ointment' (which is a mistranslation of treatment for a major blood vessel; Cule, 1966) and four pence for an application of herbs for a swelling. The most famous of these men was Rhiwallon, physician to Prince Rhys Gryg, Lord of Llandovery and Dynevor Castles. He and his three sons, Cadwgan, Gruffydd and Einion, are known as the 'Physicians of Myddfai' in Carmarthen. They made a collection of herbal recipes for general illness and for many skin disorders, which had been used at the Court of Hywel Dda and before. Direct descendants of the Myddfai physicians practised medicine until 1739, when Jon Jones, the last lineal descendant of Einion, died. The modern book, 'The Physicians of Myddfai', was written in Welsh, was compiled by William Bone of Llanpumpsaint and preserved in his family until 1800, and was translated in 1861 into English by John Pughe, FRCS, of Aberdovey. The first part is probably authentic and dates from the thirteenth century. Treatments for eczema, impetigo, warts, smallpox,

FIGURE 3. King Hywel Dda, 'Howell the Good'. Reproduced by permission of the Wellcome Institute of the History of Medicine from *Ancient Cymric Medicine.*

ringworm and leprosy are included. The origin of the Myddfai Physicians is also given in the book as the 'Legend of Llwyn-y-fanbach'—the 'Lady of the Lake' in Carmarthen.

The Legend of the Lady of the Lake

The son of a widow sees the beautiful fairy of the Lake, Rhiannon, and seeks to marry her. Her fairy father makes him choose her from her beautiful sisters, all of whom look alike. He can do this only when she puts her foot forward to identify herself. She consents to live with him so long as she is not given three 'causeless blows' ('Tri ergyd diachos'), such as a playful tap on the shoulder, or, in another interpretation, so long as she is not touched with iron (i.e. the Celtic iron feared by the earlier Cymry). It was inevitable that she should vanish back into the lake, but she reappeared to teach Rhiwallon, her son, the healing herbs.

MEDIEVAL WALES

Now began the disintegration of the early Welsh kingdom. The Norman wars in Wales (A.D. 1087–1150) are obscure but are recorded in ballads about King Arthur and Merlin and the quest for the Holy Grail, although it seems improbable that Arthur lived in Wales (Williams, 1941), and in tales recorded by Lady Charlotte Guest as told by story-teller bards (Cyfarwydd) (in the *Mabinogion*, Jones & Jones, 1957) who flourished up to the fifteenth century. Then followed two wars of independence, and Llewellyn ap Gruffyd (*c.* 1258) was the first to style himself the Prince of Wales; the second later became Edward II. Owen Glendower (A.D. 1400–1415) (Rees, 1972), seeking aid from France, made sorties from the heights of Plynlimon mountain. He established his authority over all Wales and made a parliament at Machynlleth until Henry Tudor (Henry VII), bearing the standard of the red dragon at the battle of Bosworth, ascended the English throne, and made a union of the two kingdoms of England and Wales. Henry VIII (Fig. 4), however, by the Act of Union (1536), finally destroyed the Welsh kingdom by incorporating all Welsh counties into England. University education in England was available at Oxford and Cambridge, from the thirteenth century, and also elsewhere in Europe, but not within Wales. Under the Normans the Welsh holy wells became dedicated to other saints. Some curative wells treated leprosy (clafr) but there were also established church lazar houses and, in Cardiff, a leper hospital founded by the townspeople stood near the site of the old Royal Infirmary (Cule, 1970). The 'Black Death' plague (1348–9) decimated both the lowland Englishry and the upland Welshry (Ziegler, 1969).

By the sixteenth century there were two distinct medical traditions in England and in Wales (Williams, 1928). The educated University graduate migrated to the towns to treat the upper and the middle classes, and the ignorant village leech, or itinerant quack, versed in druid herbs and Anglo-Saxon magic, treated the country folk and the poor. The holy wells of medieval Wales became the doctors of the poor (Jones, 1954). Many wells had dermatological reputations. At some, warts were pricked with pins and needles, which were then thrown into the well. Others cured wens, sore legs, erysipelas, cancers and sores. In Denbigh, at Fynnon Ddyfnog, special rooms were provided for cure of scabs, the itch, and 'some said it cured Pox' (Jones, 1954). Written contributions in dermatology began to be made by Welsh doctors, who had been educated in law, theology and other disciplines, and also by rich landowners who collected manuscripts. Thomas Phaire (1955), who died 1560, wrote the first book on paediatrics in English, the 'Boke of Chyldren'. He recorded such skin diseases as 'Scalles of the head', 'Scabbyness and ytche', 'canker in the mouth', 'failyng of the skynne' and 'chafing of the skynne', 'small poches and measles', 'sacer ignis or chingles', and 'swellyng of the head' as from the sides of bacon or salt beef falling from hooks in the ceiling, as well as stiffness of the limbs as when a child is found in the frost or street cast away by a wicked mother; which gives some idea of the then current social conditions.

Humphrey Llwyd of Denbigh (1568), a geographer, translated the 'Thesauris Pauperium', as the 'Treasury of Health', recommending arsenic for curing 'Wyddfire' or ringworm, and David Samuel (1751–1798), travelling with Captain Cook, described scurvy and syphilis in the Sandwich and Friendly Isles (Wellcome Historical Medical Museum, 1928). There were also leech books compiled from the old manuscripts, such as Hafod 16 (Jones, 1955, 1956, 1958, 1959) and 'the Welsh Leech book', (Llyfr o Feddyginiaeth) (Lewis, 1914). Herein are treatments for erysipelas, boils, bites, pockmarks and scales. Lice were removed by grinding wild sage with a pennyworth of mercury and superfluous hair by nettles boiled with vinegar.

FIGURE 4. King Henry VIII, by Holbein. Reproduced by permission of the City of Liverpool Walker Art Gallery

MODERN WALES (17TH–19TH CENTURIES)

After the Reformation, changes in religion, the awakening of scientific teaching and the coming of industry significantly changed Wales and its dermatology. Non-conformist doctrines led to the destruction of Roman Catholic churches and effigies, with impairment of established well cults. Pilgrimages still continued to some wells, such as St Winifred's at Holywell (Jones, 1954). The scientific possibility that chemicals in the water, instead of miracles or magic, might be responsible for cures encouraged analysis of the waters. After the discovery of minerals, curative spas began to be established at Llandrindod Wells and Llandudno. The Reverend Theophilus Evans, with scurvy, was encouraged to bathe at Llanwrtyd Wells after seeing a very healthy frog emerge (Jones, 1954). 'Black sulphurous mineral springs' in Llanbister, Radnorshire (Williams, 1905), and elsewhere were famed for the cure of 'cutaneous distempers'. The Industrial Revolution in Wales in the eighteenth and nineteenth centuries, with its coal mines and steel works (1750), lead and copper (1800–1850), wool and slate (1850) (Rees, 1972), led to canals and railways, and to larger towns of coal miners and steel workers. Dermatology was part of the medical care of workers by truck doctors (payment of wages in goods or cheques valid in stores owned by the employers) and later by practitioners on contract to employers.

Occupational cellulitis in colliers later so increased that the Medical Research Council eventually produced a report on cellulitis and bursitis in 'beat knee, beat hand and beat elbow' (Collis & Llewellyn, 1924) in Wales and elsewhere.

The intellectual leaders of Wales in the seventeenth to nineteenth centuries were still hymnists, preachers, musicians, poets, bards and revivalists, living in houses isolated in the valleys (Edwards, 1931). A former Welsh prime minister, David Lloyd George (1912), in the House of Commons described vividly the situation even in the twentieth century. 'Anyone who has lived in these mountains knows what sickness means. There are miles of track broken and rutted by the winter rains before you reach the high road. The people there never send for medical aid for petty ailments. He is not even summoned for important family events'. The village old lady, with her herbal expertise, was thus often the dermatologist.

YESTERDAY AND TODAY

The dream that Wales might have its own university was not fulfilled until its colleges began in Aberystwyth 1872, in Cardiff 1883, and later in Swansea and Bangor. Preclinical medical teaching was available at the Cardiff Royal Infirmary from 1893 to 1921, in which year a medical degree M.B., B.Ch. (Wales) was instituted (Jones, 1971).

Probably one of the first persons with training in dermatology to practise in hospital in Wales in the present century was Dr Owen Rhys at the Cardiff Royal Infirmary (Glamorgan), but the first full consultant dermatologist to be appointed was Dr Percy Ingram at the Royal Gwent Hospital in Newport (Monmouth).

Owen Llewellin Rhys M.D. B.Ch.(Edin.) (1875–1963) (*Fig.* 5)

Born at Neath, South Wales, he qualified at Edinburgh Royal Infirmary. He learnt his dermatology as a house physician in the skin department. In 1901 he was appointed resident medical and surgical officer at the Cardiff Royal Infirmary. His later predominant interest in radiology was aroused by treatment of simple skin disorders including tinea capitis by X-ray, with experience gained under the tutelage of Dr Thurston Holland, the pioneer radiologist in Liverpool. He became in 1906 medical officer in charge of the 'electrical pavilion' at the Infirmary, a name which still adorns the door of the old department of dermatology, and later lecturer in radiology to the Welsh National School of Medicine.

FIGURE 5. Dr Owen Llewellin Rhys.

His writings were on radiological subjects (Rhys, 1934) rather than on skin disorders. As a territorial soldier he served in the 2nd Welsh Field Ambulance in the 1914–18 war. When president of the Cardiff Medical Society (1930) he was host to the eminent dermatologist Dr Graham Little who, as MP for London University, was his chief guest.

Cecil Parker Ingram M.R.C.S., L.R.C.P.(Lond.), M.B., B.S.(Lond.) (1875–1955) (*Fig.* 6)
Dr Ingram was educated at Guy's Hospital, qualifying in 1899. He was honorary physician in diseases of the skin and medical officer in charge of the venereal diseases department at the Royal Gwent Hospital, Newport, from 1920 to 1945. Later he was consultant dermatologist also to Blaina and District general hospital and to Woolaston House Emergency Medical Service. Coming under the influence of Colonel L.P. Harrison, the eminent venereologist, his contributions were mostly on venereal diseases (Ingram, 1926, 1930, 1932). He was a member of the British Association of Derm-

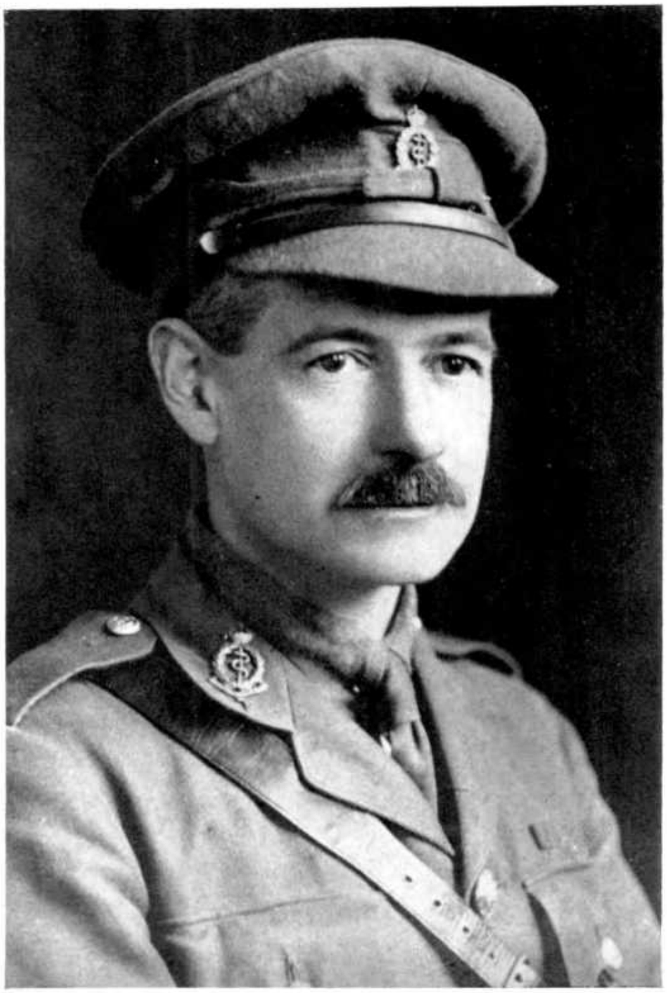


FIGURE 6. Dr Cecil Parker Ingram.

atology, and a medical referee for dermatology cases. He had the honour of being commissioned as a deputy lieutenant for the county of Monmouthshire (1941).

Ointments with many ingredients were then individually compounded and dispensed. A famous Cardiff chemist's shop was founded by Robert Drane F.L.S., first in Bute Street (1857) 'under the delusion that it must become the principal street in the town' (Annotation, 1901). It was recommenced in Queen's Street in 1868 when there were three pharmaceutical chemists in the town, and when the Pharmacy Bill, setting up a register of pharmaceutical chemists, chemists and druggists, became law. It continued to flourish during the first half of the twentieth century under the late Mr Alex. Johnson. The reliance of many patients from the Rhondda valleys upon 'Drane's ointments' for skin disorders is well within the memory of many today.

The Welsh National School of Medicine was born in 1931. Dermatology still continued to be combined with venereology.

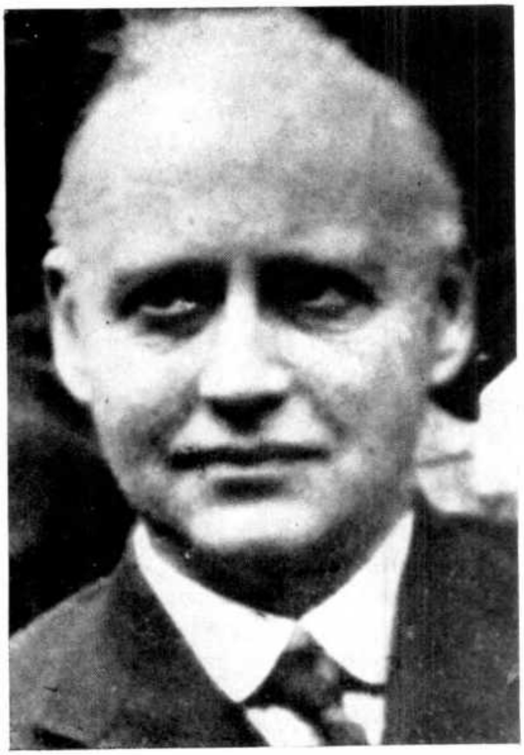


FIGURE 7. Dr James Beatty.

During the next two decades, leading to the second world war (1939–45) and the introduction of the National Health Service (1948), other dermatologists were appointed in the Principality.

In Cardiff these were:
James Beatty M.B.(Lond.), M.R.C.P.(Lond.), D.P.H. (1895–1947) (*Fig.* 7). When he was appointed the first honorary dermatologist at the Cardiff Royal Infirmary (1923–1937) and later lecturer in dermatology and in pharmacology, he initiated the present dermatology department of the Welsh National School. He was also dermatologist to the Queen Alexandra Hospital, Weston-super-Mare. He trained in Dublin, and later in London, with honours and the gold medal for anatomy. He was Vice-President of the section of Dermatology of the Royal Society of Medicine in 1928 and 1934, and President of the Cardiff Medical Society 1940–41. During the war years he came back from retirement to stand in for his successor at the Infirmary, Dr Francis Bettley, who was on active service. Many remember his ability to diagnose common skin diseases with considerable accuracy by touch when his sight was failing.

Francis Ray Bettley. He followed Dr Beatty in 1937 as Consultant Dermatologist at the Royal Infirmary and lecturer in dermatology, and resigned in 1946 to go to the Middlesex Hospital in London.

In Swansea there was:

David Rhys Lewis M.D., B.Ch.(Edin.), F.R.C.P.(Edin.) (1890–1957). He was the first visiting physician for skin disorders on the staff of the Swansea General Hospital (1930–1957), and in 1935 was also made medical officer in charge of the VD department at Swansea. Graduating in Edinburgh in 1920, he trained in dermatology in Paris and Vienna. He was clinical assistant at the skin department in Cardiff, 1928–29. He started practice as a general practitioner in Llansamlet near Swansea and, consequent upon his experience with the tin workers in this industrial area, 'industrial dermatitis from radiant heat' was later included in the schedule of disorders under the workman's compensation act. He had an exceptionally large 'parish' for any dermatologist and throughout Wales, where so many have the same surname, he was identified affectionately as 'Dai skins'. He became in turn dermatologist to Pembroke Memorial Hospital (1934), West Glamorgan Hospital, Neath, Gorseinon and Morrison Emergency Hospital (1935), Llanelly General Hospital (1943) and Aberdare hospital (1945). In 1950 he became a part-time consultant dermatologist under the NHS based on Swansea General Hospital, and was asked to continue 1 year after reaching the age of retirement at 65.

A patriotic and knowledgeable Welshman, he was very honoured by his election to the Court of Governors of the National Library of Wales and by being made High Sheriff of Breconshire in 1950.

At Bangor: *Dr Dorothy Lancaster (nee Godden)* (1900–1961) (*Fig.* 8). was made physician with care of the female venereal disease department and temporary war-time dermatologist in 1944, before being appointed in 1946 as the first consultant dermatologist at the Caernarvon and Anglesey General hospital at Bangor. She started the first skin department at this hospital and later in the Clwyd and Deeside area at the Royal Alexandra Hospital, Rhyl. She trained at the London School of Medicine for Women, at St Mary's Hospital in 1929, and later studied dermatology under Oppenheim in Vienna for 1 year. She worked in 1930 in the Special Department in Bristol under Dr Kenneth Wills. She was a great supporter of the Medical Women's Federation, serving as its secretary and president, and she gave an address on skin disorders at their International Conference in Italy in 1954. She was president of the Caernarvon and Anglesey branch of the Royal College of Nursing, the Bangor Inner Wheel and Soroptomists Club.

Since the expansion of the National Health services, many more dermatologists have been appointed throughout the Principality. These are, in Wrexham, Netta Hay (1940), in Newport, Dr Bernard Thomas (1945), and in Cardiff, G. A. Hodgson (1946), A. J. Rook (1950), who later moved to Cambridge (1953) and was succeeded by E. Waddington (1953). Elsewhere there were, in Swansea, Leighton Rees (1957), in Neath, Roderick Howell (1958), in Clwyd and Deeside based on Rhyl, Ellen Emslie (1962), in Pontypridd, Charles Long (1965), in Bangor, William Beer (1965), and in Llandrindod Wells, Ann Lawton (1967).

The present University Hospital of Wales was commissioned in 1965 and opened by H.M. the Queen on 19 November 1971. The dermatology department was transferred from the Cardiff Royal Infirmary, and the dermatology ward was one of the first two wards to be opened on that day. The first patient, who was specially chosen to be admitted to the University Hospital of Wales, suffered from psoriasis! The recent academic appointment of a senior lecturer, Dr Ronald Marks, in July 1973, begins a new stage in the story of dermatology in Wales.

ACKNOWLEDGMENT

I wish to record my thanks to Dr John Cule, Lecturer in the history of medicine to the University of Wales in Cardiff, for help in the preparation of this article.

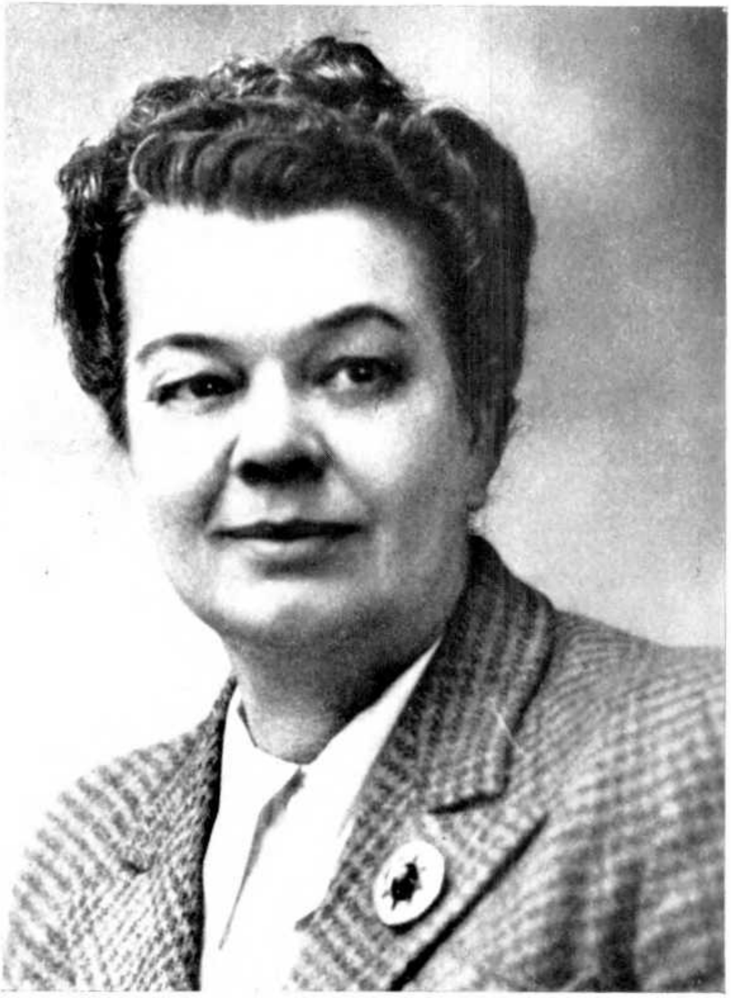


FIGURE 8. Dr Dorothy Lancaster.

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Comment

TRICHOMONIASIS AND THE DERMATOLOGIST

Specific vaginitis due to *Trichomonas vaginalis* is fairly common in women during the sexually active period; one estimate is that over a million women are affected in the United States of America. In addition, *T. vaginalis* may be present with few or no symptoms, especially in older women. In men *T. vaginalis* can give rise to urethritis, prostatitis and occasionally balanitis, but its incidence is probably low. Some venereologists believe trichomonas vaginitis to be always sexually transmitted, but one must express doubt on this, as the organism is capable of surviving in adverse conditions outside the body for up to 24 h, for example in properly chlorinated water of swimming pools; therefore non-sexual transmission is a strong possibility.

In this country venereologists generally consider that *T. vaginalis* is confined to the lower urogenital tract and may cause vaginitis, vulvitis and sometimes intertrigo and excoriation of the skin of the neighbouring thigh, but no systemic or metastatic lesions. This attitude is also reflected in our current textbooks. Thus trichomonal skin involvement as recognized in this country is minimal and localized and clears rapidly once the vaginitis is controlled. Yet from the continent of Europe a number of reports suggest that *T. vaginalis* may induce a variety of systemic lesions affecting the skin, the mucous membranes of the mouth, the joints, the liver, the gastrointestinal tract and the kidneys. In a recent monograph by Kolman (1973), a series of case histories is presented of women with trichomonas vaginitis, who had also one or more of the following lesions: eczema, itchy dermatoses of the limbs, extragenital pruritus and oral aphthosis. Apart from skin lesions there were acute or subacute arthritis not responding to antirheumatic drugs, abdominal symptoms of gastritis or colitis, and anxiety and/or depression. Some patients appeared to have renal involvement and were also found to have hepatosplenomegaly. Treatment with the antitrichomonal drug metronidazol (Flagyl) was apparently effective in clearing the genital and extragenital lesions, though arthritis responded only in the early stages. Surprisingly, a number of these patients were menopausal women in whom trichomonas vaginitis was for long not suspected. There are only few comparable reports of men believed to have systemic *T. vaginalis* involvement.

What are we to make of this difference in the experience in this country and across the Channel? The claim that the extragenital lesions, including those of the skin, cleared fairly promptly with oral metronidazol is a strong point in favour of their being directly or indirectly due to *T. vaginalis*. Against accepting this view on the evidence offered is the sketchiness and lack of critical assessment of many of the case histories in this monograph, as in other reports. Kolman believes that the systemic lesions are due to a hypothetical toxin elaborated by the protozoon, as *T. vaginalis* has not been found in any of the extragenital lesions. As mentioned, trichomonas vaginitis is common and the presence of various skin lesions, arthritis or gastrointestinal symptoms in the same patient does not necessarily indicate a causal relationship. Kolman is perhaps on safer ground when he observes that *T. vaginalis* is often missed and that there is a case for more frequent and painstaking examination for the vaginal parasite, especially as the treatment is simple, non-toxic and most effective.

One's own impression is that there is insufficient scientific ground for assuming the existence of systemic trichomonas lesions in man, but that one should not ignore this possibility out of hand and that we should investigate any case in which this association appears to be even remotely likely. A study in its simplest form should include strictly controlled therapeutic trials with anti-trichomonal drugs.

Watford General Hospital,
Shrodells Wing, Vicarage Road, Watford

G.W.CSONKA

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Obituary

A.C.DEWAR

Alisdair Campbell Dewar died in Glasgow on 2 December 1973, a few days before his 76th birthday, after a lifetime of indifferent physical health, the discomfort and fatigue of which were borne privately and uncomplainingly.

Soon after he was born the family moved from the south side of Glasgow to the Lambhill district on the north side, where his father managed two branches of the Royal Bank of Scotland. Alisdair's daily journeys to Hutcheson's Boys' School were punctuated by visits to the Municipal Library to read his favourite magazines, but his schooldays were more notable for the life-long friendships formed than for academic prowess. The first world war broke out shortly after he had started medicine at Glasgow University. He volunteered immediately for army service but was found medically unfit; but after passing his second M.B. he managed to get himself accepted as Medical Officer aboard a mine-sweeper, where he served 3 years.

Having qualified M.B., Ch.B. in 1921, he worked at Barnhill Hospital (now Foresthall), where he took his D.P.H. He then started on a period of 25 years spent in general practice at West Princes Street, Glasgow. His kindness, compassion and perseverance combined to produce an effective and trusted family physician, who was known to turn a willing ear to any call for help.

Advised early in his career to cultivate a special subject, he chose dermatology, and studied with Dr Wyllie Nicol at the Western Infirmary. He became Dr J. Ferguson Smith's assistant at the Royal Infirmary in the late 1920s, in which hospital he continued to work until he retired in 1962. He originally combined dermatology with general practice, as was the custom, but with the advent of the National Health Service he was appointed part-time consultant dermatologist, and from then on devoted himself entirely to dermatology. His health declined further. As long as he could stand he refused to stay off duty, but he was forced to spend most of 1944 in hospital and another 6 months in bed in the early 1950s. To look at the man you would have thought he was dying, but he lived on to the astonishment of his colleagues, and to their admiration too at his tenacious hold on life.

He had a general interest in clinical dermatology, especially in lupus vulgaris, but he was always attracted more to the personalities of his patients than to the diseases from which they suffered. He lives on in their hearts and also in the memory of his students, for he loved teaching. He and his wife supported the University tutorial scheme enthusiastically and they are remembered gratefully to this day by the students who were lucky enough to fall under their influence. A good talker, a good listener, fond of music, the theatre, art and history (especially of Glasgow and of Scotland); in his younger days a yachtsman; a founder member of the Saltire Society; politically tolerant, he perhaps thought of himself as an old fashioned liberal. He married in 1936 Mary Howat Bennett, who survives him and with whom, in spite of illness, he forged a life of quiet contentment. Their son Donald is a solicitor and an ex-M.P.

Alisdair Dewar endured with dignity, and was gentle and considerate to his fellow men.

J.O'D.ALEXANDER
A.LYELL

Correspondence

PROTEIN-LOSING ENTEROPATHY IN DERMATITIS HERPETIFORMIS

SIR, The case report of this condition by Toft, Heading & Simpson (1973) has many similarities to a patient recently investigated by us. As only two other instances are recorded in the literature (Gjone & Oyri, 1970), we felt that it would be useful to add a further comment on this facet of dermatitis herpetiformis enteropathy.

D.L., a 56-year-old insurance clerk, was seen initially in August 1967 with a rash of 3 months' duration which in clinical features, histology and response to dapsone was typical of dermatitis herpetiformis. While taking a maintenance dosage of 50–150 mg/day of dapsone, he was referred back to the skin clinic in August 1972 with massive and bilateral leg oedema of several months' duration. He was a slightly built man, weighing 59 kg, who had observed no weight loss and who had no gastro-intestinal symptoms. His only complaint was of some tiredness. Physical examination was normal apart from gross pitting oedema to knee level, and a mild anaemia clinically.

Investigations were as follows: Hb 12·1 g%; ESR 5 mm in the 1st h (Westergren); serum albumin 1·7 g/100 ml and globulin 1·6 g/100 ml; serum IgG 625 mg/100 ml (normal 800–1800), serum IgA 190 mg/100 ml (normal 90–450), and serum IgM 50 mg/100 ml (normal 60–250); serum vitamin B_{12} 305 pg/ml (normal 170–800); serum folates 2·5 ng/ml (normal greater than 3); serum iron 25 μg/100 ml (normal 65–175) and iron combining capacity 192 μg/100 ml (normal 250–420); serum cholesterol 127 mg/100 ml (normal 140–180). Normal results were obtained for liver function tests, serum electrolytes and blood urea. The urine contained no abnormal constituents. Over a 3-day period, faecal fat excretion was 21·3 g/24 h; the stools contained no occult blood. An upper jejunal biopsy taken with a Crosby capsule showed a flat mucosa infiltrated by plasma cells and lymphocytes. ^{51}Cr-chloride was used to assess gastro-intestinal protein loss and over a 7-day period the mean daily excretion in the faeces was 2·1% of the intravenously administered dose (normal: less than 0·2% per day). A barium meal and small bowel follow-through demonstrated a 'malabsorption pattern' of flocculated barium and dilatation of bowel loops.

A gluten free diet was instituted, and after 6 months' reinvestigation showed continued serum folate deficiency (1·9 ng/ml), a diminished but still abnormal fat excretion (13·7 g/24 h), a further fall in IgG (360 mg/100 ml), an unchanged serum albumin, but a further increase in the faecal excretion of intravenous ^{51}Cr-chloride (3·5% per day). No weight had been gained, his requirement for dapsone was unchanged but tiredness was less. He was enthusiastic to continue his gluten free diet but would not consent to further jejunal biopsy.

The enteropathy of dermatitis herpetiformis has many points in common with that found in adult coeliac disease. The villous changes of the small intestine in coeliac disease are found in varying degree in over two-thirds of patients with dermatitis herpetiformis (Marks, Shuster & Watson, 1966), and, while the skin disease is rarely associated with gastro-intestinal symptoms, there is an appreciable incidence of increased faecal fat excretion and deficiencies of folate, iron and IgM (Fry *et al.*, 1967). In dermatitis herpetiformis enteropathy, a gluten free diet reverses the intestinal abnormalities and improves the diarrhoea and steatorrhoea in most instances (Shuster, Watson & Marks, 1968).

The incidence of hypoproteinaemia varies in the two diseases, however, and the paucity of reports of intestinal protein loss in dermatitis herpetiformis enteropathy is in sharp contrast to adult coeliac disease. Hypoproteinaemia was found in over 50% of cases by Green & Wollaeger (1960), the abnormality probably representing the interaction of diminished amino acid absorption, hepatic dysfunction and intestinal protein loss. Parkins (1960) used radio-iodine-labelled polyvinylpyrrolidine to demonstrate protein losing enteropathy in four of eleven patients with idiopathic steatorrhoea, and Van Tongeren (1967), using ^{51}Cr-chloride, reported abnormal protein loss in all four cases of adult coeliac disease. A comparison of intestinal protein loss in the two enteropathies is difficult to make, however, since the investigation is not routinely performed in dermatitis herpetiformis. If a true difference does exist then it may be due to the limited intestinal involvement found in some instances of the skin disease. Brow *et al.* (1971), using the technique of multiple intestinal biopsies, have commented on the patchy distribution of proximal intestinal lesions in six out of twenty-one cases of dermatitis herpetiformis enteropathy. They contrasted this with the more diffuse involvement usually found in adult coeliac disease. When limited intestinal involvement occurs in dermatitis herpetiformis it could explain the rarity of symptoms and variability of biochemical evidence of malabsorption.

We recognise the difficulty in diagnosing lymphoma of the small bowel as a complication of coeliac disease and as a cause of protein losing enteropathy. When lymphoma complicates adult coeliac disease (Harris *et al.* 1967), the changed symptomatology includes abdominal pain, bowel perforation, increased diarrhoea, weight loss and fever. There was a mean duration of 0·4 years between the onset of these symptoms and the establishment of the diagnosis. Our patient has had intestinal protein loss for at least 18 months and his clinical state has improved.

Toft *et al.* (1973) reported some decrease in intestinal protein loss 5 months after the institution of a gluten free diet. The patient whom we describe had a gluten free diet for 6 months, and while maximum improvement may take longer, he resembles one of the patients reported by Gjone & Oyri (1970), in that a gluten free diet produced improvement in steatorrhoea but had no effect on intestinal protein loss.

Skin Department,
Norfolk and Norwich Hospital,
Norwich

A.W.McKENZIE
J.R.RANDAL

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ANOGENITAL HERPES ZOSTER IN TWO CASES OF WEGENER'S GRANULOMATOSIS

SIR, The recent article by Peter D. Fugelso *et al.* (1973) on herpes zoster of the anogenital area affecting urination and defaecation encourages us to report two cases of Wegener's granulomatosis with anogenital herpes zoster, seen at our Department of Dermatology, Sahlgren's Hospital, Gothenburg, one in April and the other in October 1973. Both patients had been treated with chlorambucil and prednisolone. The cases reported by Fugelso *et al.* had no associated disease of importance. Wegener's granulomatosis is not known to predispose to herpes zoster.

Case 1: A 54-year-old automobile test driver had suffered from Wegener's granulomatosis since 1967. He had been treated with prednisolone since 1968 and with chlorambucil since 1970. He was admitted to hospital because of necrotic herpes zoster of the left buttock, the back of the left leg, and the left side of the scrotum and penis, in an S1–S4 dermatome distribution. Retention of urine necessitated catheterization. Cystoscopy demonstrated vesicle formation in the bladder. Recovery was uncomplicated and the catheter could be withdrawn after about 1 month.

Case 2: A 73-year-old woman had suffered from Wegener's granulomatosis since 1971. Between 1971 and September 1973 she was treated with chlorambucil and prednisolone. During the 2 months before admission she had been treated with azathioprine. She was admitted to hospital because of gangrenous herpes zoster in an S2 distribution. She developed urinary incontinence. Cystoscopy was not carried out. Very slowly the incontinence and the skin changes recovered.

The observation of two cases of Wegener's granulomatosis with herpes zoster, in a relatively short period of time, is remarkable in view of the low incidence of Wegener's disease. The importance of this disease, or more probably the treatment prescribed, in the development of anogenital herpes zoster should be considered.

G.ROUPE

Department of Dermatology,
Sahlgren's Hospital,
S-413 45 Gothenburg,
Sweden

REFERENCE

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Book Reviews

Recent Advances in Dermatology. No. 3. ARTHUR ROOK (Ed.) (1973) Edinburgh and London: Churchill Livingstone. Pp. ix+469, figures and tables. Price £7.50.

Recent Advances in Dermatology was published first in 1936 and Dr W.N. Goldsmith was its single author. Eighteen years later in 1954 he was joined by Dr F.F. Hellier for the second edition. The prodigious increase in the volume of dermatological literature since then is well illustrated by the fact that for the third edition of this book the editor has needed no less than twenty-four authors.

The immense difficulty in selecting from the plethora of available publications is admitted by Dr Rook and he decided to solve the problem by choosing only those subjects which are either common or of special importance. His final choice of twelve is admirable and he has been equally felicitous in his choice of authors: fifteen British, five American, three Dutch and one Belgian.

Each of the twelve chapters is firmly rooted in clinical dermatology, although at the same time dealing most comprehensively with all scientific aspects of the subject. Bacteriological, fungal and viral aspects of skin disease are excellently covered in the first three chapters, although I was surprised not to see any mention of hand, foot and mouth disease. The use of DMSO as a vehicle for IDU is dealt with in the chapter on topical therapy. Then come four chapters, to which may be added the last in the book, which deal respectively with the common conditions of photodermatoses, contact dermatitis, atopic dermatitis, acne and psoriasis. It is difficult to see how any of these could have been better done, but one must congratulate Parish and Champion on their magnificent attempt to explain the pathogenesis of atopic dermatitis, and Baker and his colleagues for momentarily actually remembering the patient with a quotation from Chesterton.

There are three equally good chapters chosen for the importance rather than the frequency of the subjects, which deal with the immunology of vitiligo and malignant melanoma, immunofluorescence and electron microscopy of bullous diseases and with genetics. And finally there is a chapter on the commonest aspect of all skin disease, namely topical therapy, a somewhat banal subject which Professor Polano manages to make not only comprehensive but also pleasantly readable.

The references, each with the subject title, are arranged alphabetically at the end of each chapter, or in one case after each section of the chapter. The index is full and accurate. The publishers, too, are to be congratulated on the arresting dust-cover, as well as the paper, print and illustrations.

Some of us, with the first two editions of this publication gathering dust on our shelves, have been waiting a long time for the third. It was worth it. Dr Rook and his collaborators are to be congratulated on producing an outstanding book.

PETER BORRIE

Dermal Pathology. JAMES H. GRAHAM, WAINE C. JOHNSON AND ELSON B. HELWIG (Eds.) (1972) Hagerstown, New York, Evanston, San Francisco, London: Harper and Row. Pp. 840, 1155 illustrations, 77 in colour. Price £21.60.

This book springs from the postgraduate courses held annually at the Skin and Cancer Hospital, Philadelphia. Its seventeen contributors have produced thirty-five chapters of interest and distinction. Each is an independent commentary on some aspect of histopathology of the skin and merits consultation if reference needs to be made to the particular subject covered. Regrettably, there are many subjects left uncovered. There are no sections dealing with lichen planus, acne or rosacea for example. Some of the chapters record the author's particular interest and experience and are really research monographs. For example, the chapter on superficial fungus infection by Graham is, at 131 pages, a most authoritative and detailed work in a little explored area of dermatopathology.

It is refreshing to find that the authors do not restrict their observations to straight 'Haematoxylin and eosin' histology. The section on histochemistry by Waine C. Johnson we found particularly enjoyable, and in general, throughout the book, free reference is made when appropriate to ultrastructural findings and to enzyme histochemistry. Some of the illustrations are perhaps too small adequately to communicate their point, but overall the book is pleasingly produced. This work will be helpful to all with an interest in dermatopathology, and as it contains the views of the team from the Armed Forces Institute of Pathology this will clearly be the main value of the book.

R. MARKS
E. WILSON JONES

A Colour Atlas of Dermatology. G. M. LEVENE AND C. D. CALNAN (1974) London: Wolfe Medical Books. Pp. 368, 466 illustrations in colour, diagrams. Price £5.50.

I have some reservations about an atlas of colour photographs as a method of introducing beginners to the subject of dermatology. The authors say that the single most useful procedure in the diagnosis of skin disease is the visual inspection of the lesion. This puts history-taking into a lesser role and implies that the management of patients with skin disease should be different from that of those with other medical complaints. Having expressed what is a personal view, I cannot but admire this superb collection of colour photographs. They are so good that they will make the teaching of students and practitioners much easier.

The book starts with line diagrams of the basic skin lesions. It might be possible in the next edition to place a photograph of a similar lesion for comparison alongside the somewhat unrealistic diagrams.

There then follow chapters on the common dermatoses. For instance, Chapter 1 on psoriasis is illustrated by twenty-one pictures which cover both the common and the unusual features. Short explanatory notes appear on the lefthand page and the illustrations are mainly on the righthand page of the open book. The notes refer mostly to points of diagnosis and are fully cross referenced to aid differential diagnosis. Little mention is made of treatment.

Chapter 2 begins with a classification of types of dermatitis—a remarkable achievement to compress this into 500 words—and then follow fifty illustrations, which include some showing the techniques and results of patch tests.

Chapter 3 demonstrates infections and records syphilis in some detail. This is an important subject which is becoming again more frequent and is so difficult to demonstrate in live patients. This chapter also includes the demonstration of the method of scraping of skin and its examination for fungus mycelium with potassium hydroxide solution.

In any atlas it is always easy to criticise the selection of material and there must be a great temptation to display a collection of rare stamps. The authors in this book have struck an admirable balance; some of the rarities displayed, such as Degos' malignant atrophic papulosis and the acanthosis nigricans on the same page, are perfect reproductions which should make diagnosis simple. Ironically, the only photographs which are not quite perfect are those showing common conditions. Picture 220 of acne vulgaris is, I think, too erythematous and would be diagnosed as rosacea by most dermatologists. The gravitational ulcer in Figure 370 is so horrific as to be atypical.

I could find no misprints, the type is clear and the index full and accurate. This is by far the best collection of dermatological pictures that I have seen and it will, I am certain, become a necessity for those who teach and of considerable value to those who are taught.

I.B.SNEDDON

Antibiotika–Chemotherapeutika. Grundlagen–Anwendung–Gefahren. Ein Leitfaden für die Praxis. 2. Uberarbeitete Augeaff. H. HELWIG (1973) Stuttgart: Thieme. Pp. viii+248, tables. Price £2.20.

The second edition of this pocket book has been revised by the original author, who is a paediatrician. The book, which is of very handy format (12 × 19 cm), is clearly printed and contains a great deal of detailed basic information on the use and abuse of drugs. The concise introduction deals with the action, sensitivities, blood and tissue concentrations, spectrum of activity, side effects, dosage, indications and contraindications, and duration of treatment.

The author rightly warns against the routine use of combined preparations. He also discusses contraindications in special circumstances, such as pregnancy, the neonatal period and the presence of kidney or liver damage.

After a classification according to chemical structure and spectrum of activity, there is a chapter on indications, in alphabetical order. Proprietary preparations, mainly German and almost entirely continental in origin, are listed, with a comprehensive index of world literature.

This handbook can be confidently recommended to the German-speaking practitioner or pharmacist as well written, up to date, clearly presented and practical. Those antibiotics and other chemotherapeutic agents of any importance in dermatology are without exception competently and adequately covered.

JOHN MORGAN

Immediate Hypersensitivity. The Molecular Basis of the Allergic Response. D.R. STANWORTH (1973) **North Holland Research Monographs. Frontiers of Biology. Volume 28.** Amsterdam: North Holland Publishing Co. Pp. 400. Price £12.50.

This is the twenty-eighth in the admirable series of monographs, Frontiers of Biology, each of which deals with some fundamental aspect of biology. So far, few of these monographs have been of immediate concern to clinical dermatologists.

Dr Stanworth describes himself as an immunochemist, and it is from this point of view that this book on immediate hypersensitivity is written. There are detailed appraisals of the laboratory techniques available, so many of which the author can assess with the authority of long personal experience. Ten or 15 years ago the P–K passive transfer test was the main method of investigation available. This and the numerous and more sophisticated *in vitro* tests which have since been developed are described in detail. The chemical and physical nature of the antigens and antibodies are discussed, as are also the mechanisms by which antigen–antibody reactions cause tissue damage (or even perhaps a beneficial effect).

This book approaches the subject of atopic disorders at a fundamental level, making it evident that further research is likely soon to depart into the realms of biophysics and biochemistry. The book does not describe clinical aspects, and the practising dermatologist is unlikely to find much in it to make him alter his approach to his patients. Nevertheless, it is a masterly account of current knowledge and speculation acknowledged by immunologists and Dr Stanworth confidently predicts that within a decade such lines of study will lead to major advances in therapy.

The book is very well produced, and contains a full bibliography of some 500 references.

R.H.CHAMPION

Scleroderma: Proceedings of an International Symposium Sponsored by the World Health Organization. F.DELBARRE (Ed.) (1972) Paris: Masson. Pp. 311, illustrated. Price £13.33.

This monograph records the proceedings of a symposium at the WHO International Reference Centre for Connective Tissue Diseases in Paris in June 1969. The symposium was organized by a small group set up by the WHO in 1966, who concluded that 'research was being hindered by the lack of uniform criteria for diagnosis and classification and by differences in the techniques used for measuring some of the abnormalities in laboratory findings'.

It is doubtful whether the eleven invited members of the symposium, and the others who contributed, have clarified the problems of classification. The contributions, some in French, others in English, vary in quality. Some new material is included but many chapters only record previously published work. It is, however, useful to have this brought together in one volume. It is not clear whether there was a discussion after each contribution as only one short discussion is reported. If there was, this would have been worth publishing.

This book will be useful for workers in the field, even though the references are only up to 1968.

N.R.ROWELL

..ish Journal of Dermatology (1974) **90**, 722.

News and Notices

Investigative Group of the British Association of Dermatologists

The next meeting of the Group will be held at the Middlesex Hospital Medical School on Saturday, 25 January 1975. The meeting will be preceded by a dinner on the evening of Friday, 24 January. Members wishing to present papers should send standardized abstracts (details of which will be precirculated) to Dr M.W.Greaves, Department of Dermatology, Royal Victoria Infirmary, Newcastle-on-Tyne, not later than Monday, 14 October 1974.

Dermatopathology Symposium, New York

A symposium on dermatopathology will be held in the Postgraduate Medical School at New York University on 7–9 October 1974, under the Chairmanship of Dr A.B.Ackerman. The eighteen speakers will include Dr Ackerman, Dr Wallace H.Clark, Dr Robert W.Goitz, Dr James H.Graham, Dr John M.Knox, Dr Herbert Z.Lund, Dr Hermann Pinkus, and Dr Richard K.Winkelmann. Dr M.Judah Folkman will deliver the Howard Fox Lecture. The Chairman of the Department is Dr Rudolph L.Baer. Applications, which should be accompanied by the tuition fee of $200 ($100 for residents in approved training), should be addressed to Office of the Recorder, Room 4-44C-N, LHB, N.Y.U. Postgraduate Medical School, 550 First Avenue, New York 10016, U.S.A.

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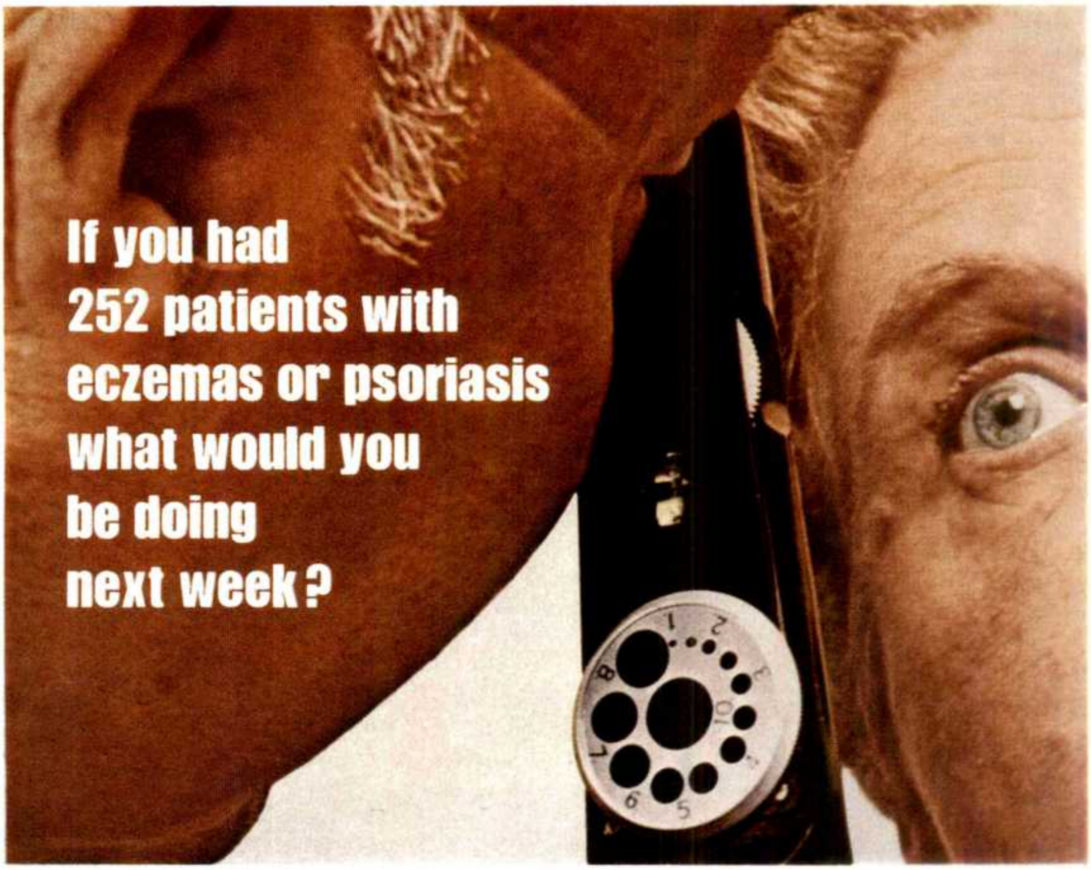

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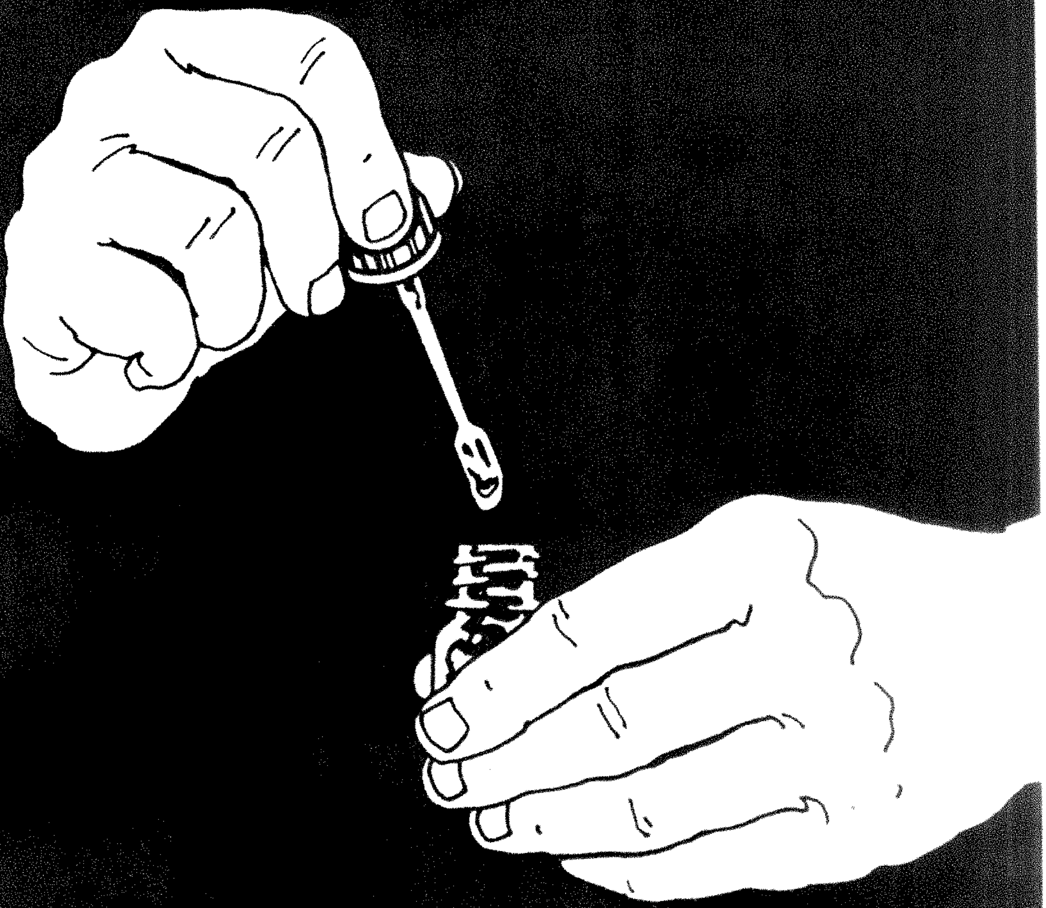

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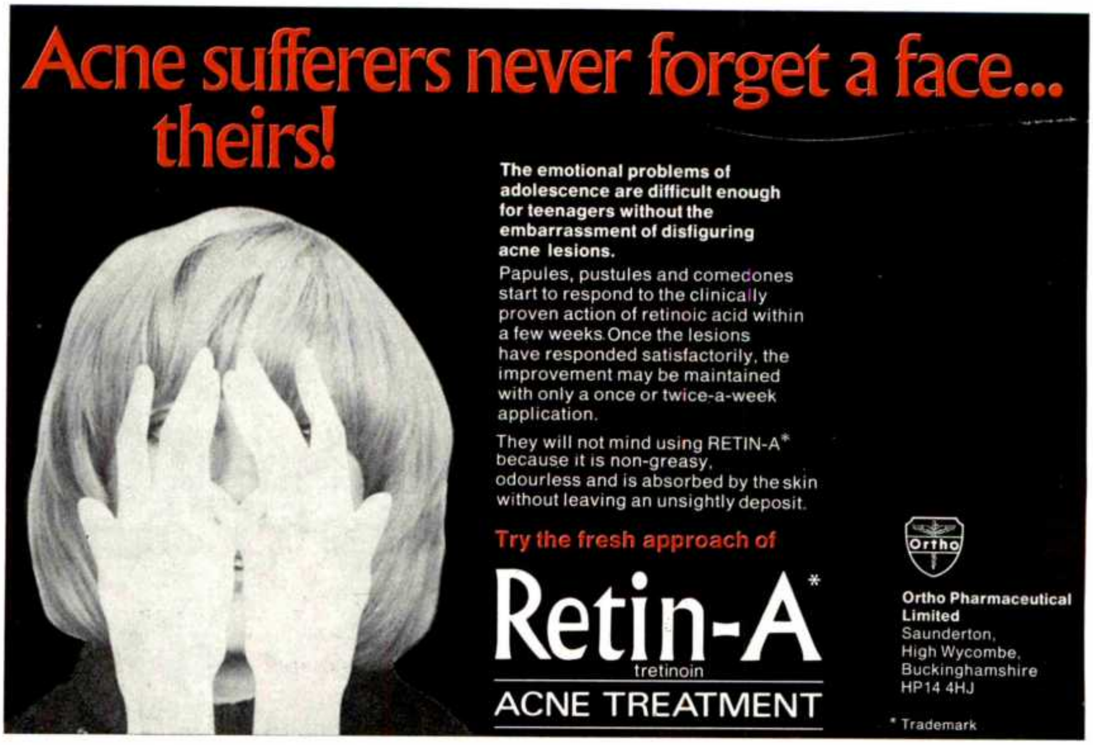
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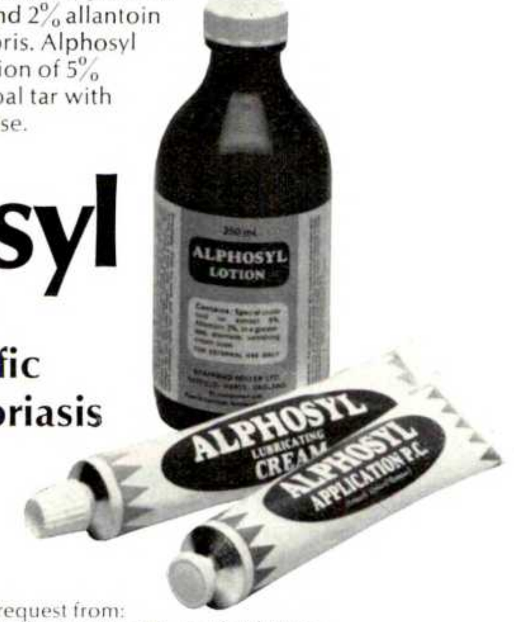

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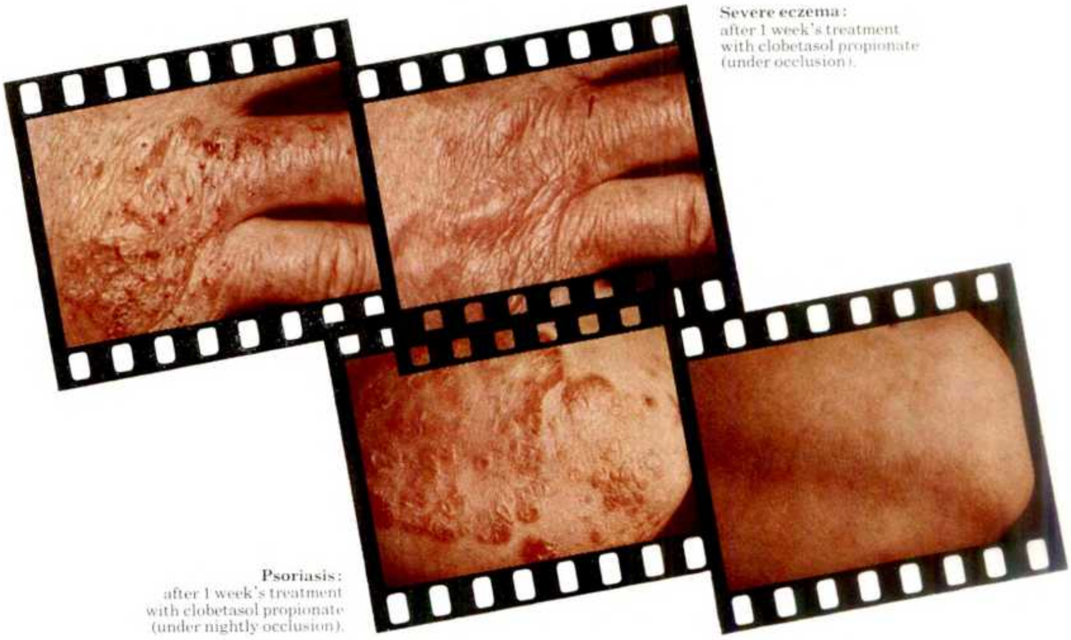

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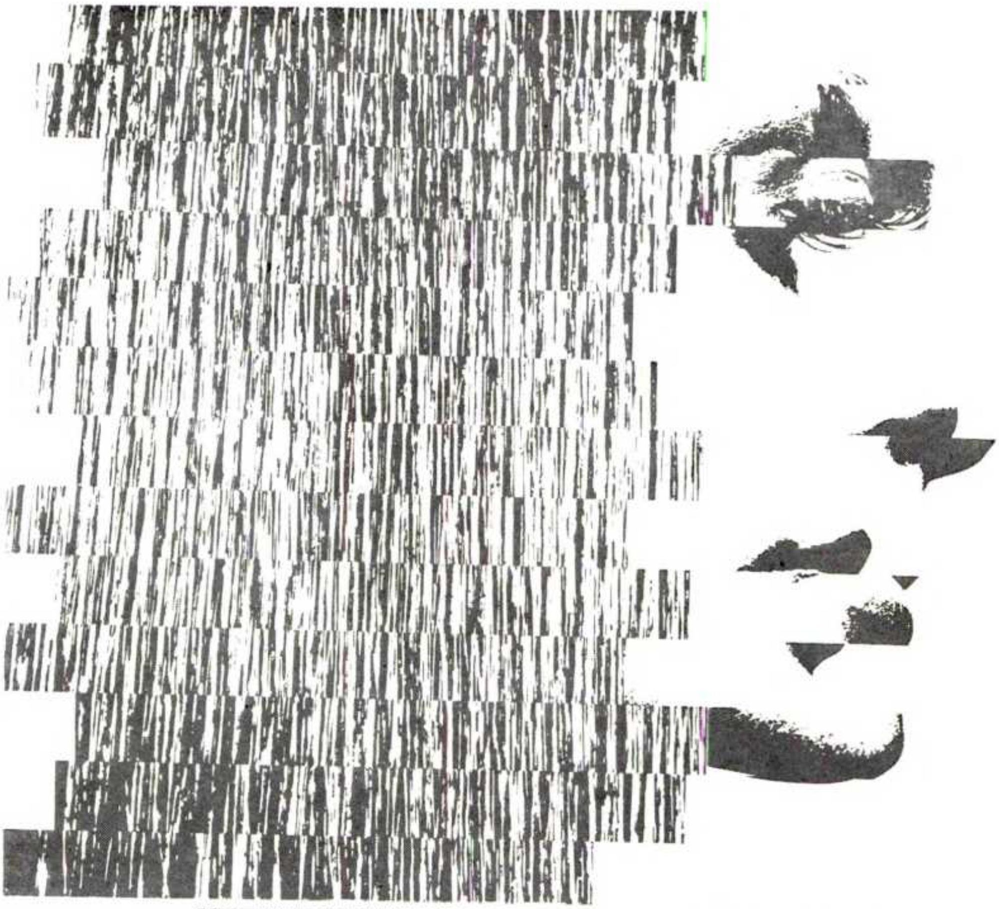

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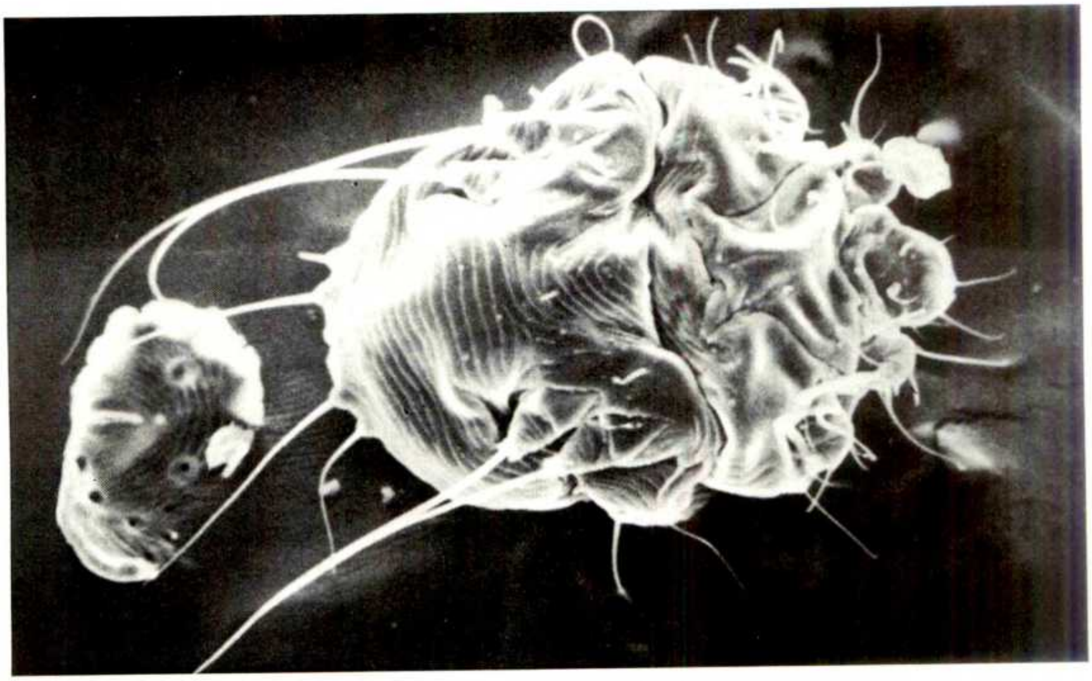

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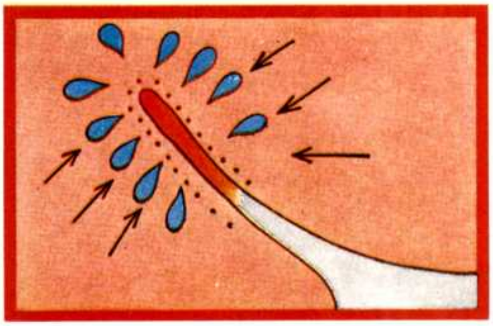

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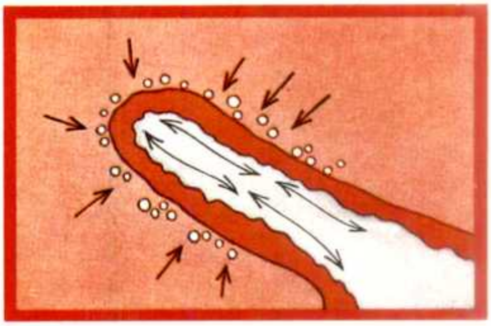

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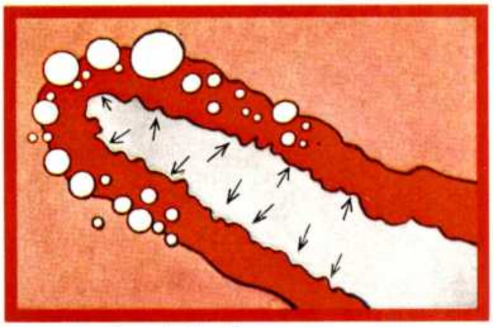

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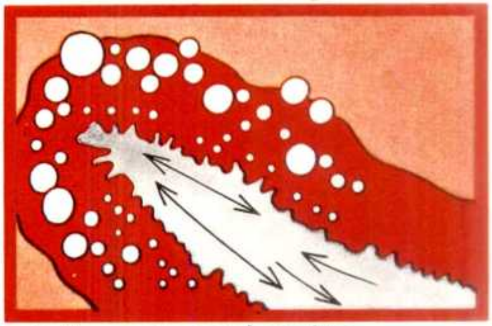

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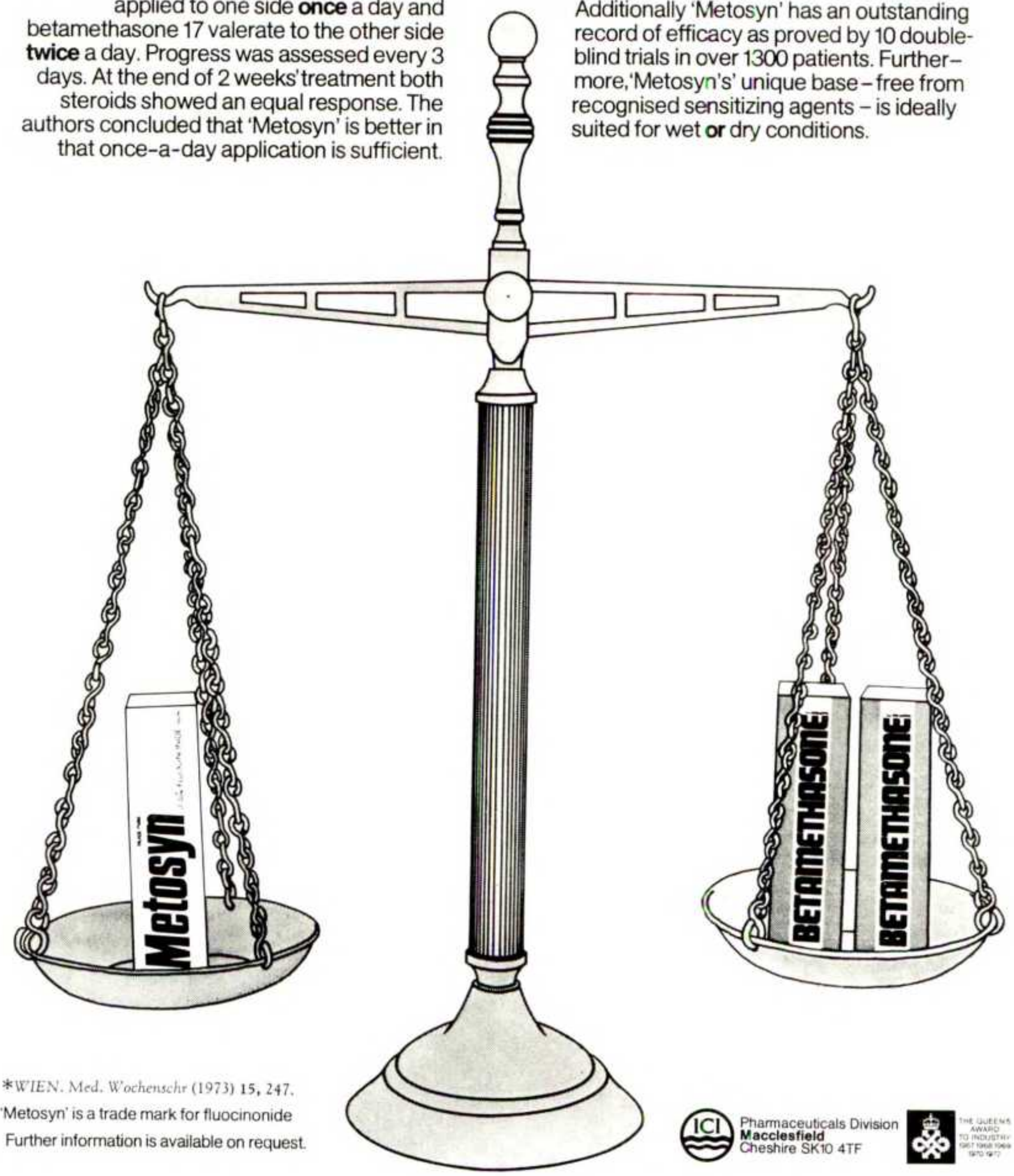

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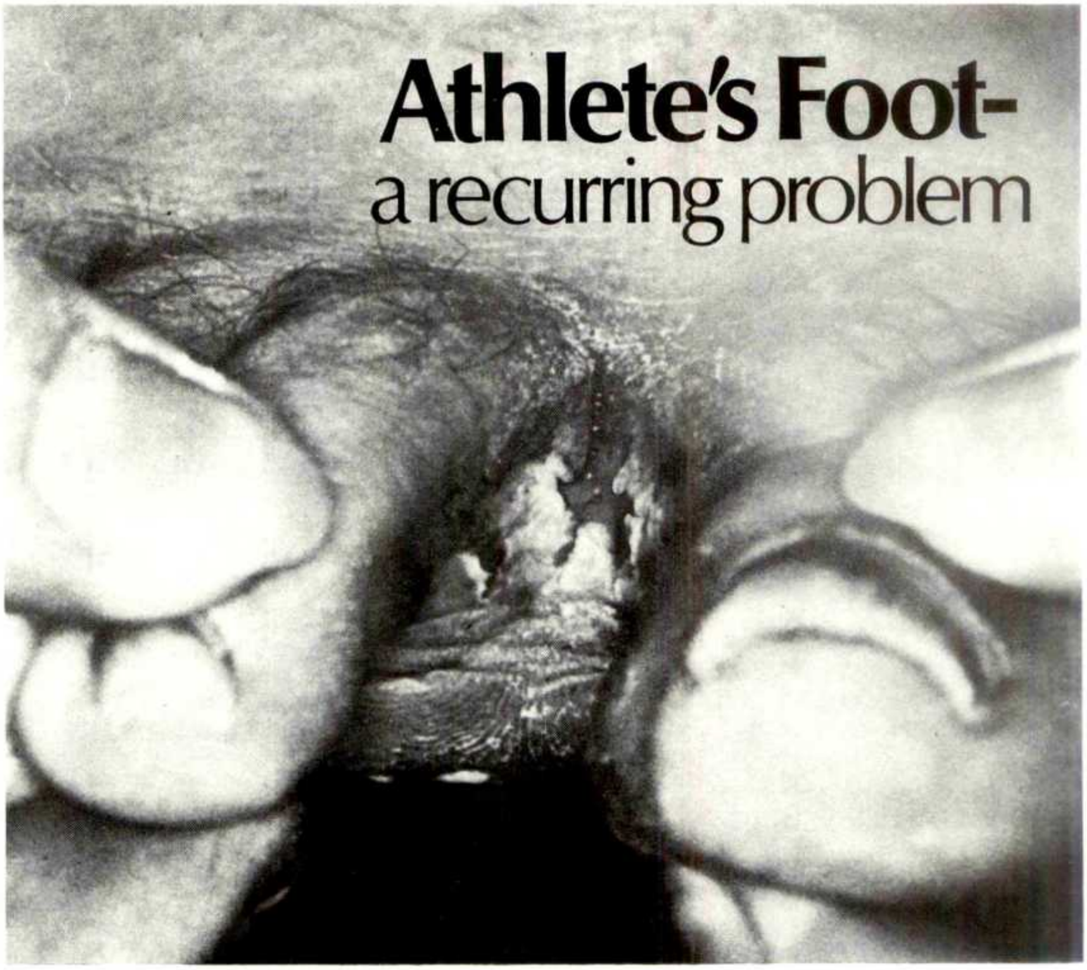

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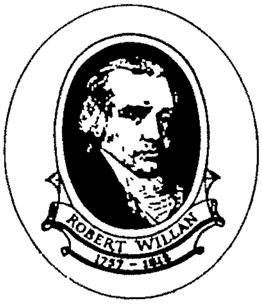

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Clinical and Laboratory Investigations

Metabolism of androgens by human skin in acne

J.B.HAY AND M.B.HODGINS

University Department of Dermatology, Anderson College, 56 Dumbarton Road,
Glasgow G11 6NU

Accepted for publication 17 January 1974

SUMMARY

Shoulder skin from young men with and without acne was incubated *in vitro* in Krebs Improved Ringer I medium with 7α-^{3}H dehydroepiandrosterone, 7α-^{3}H androstenedione and 7α-^{3}H testosterone. There were no marked differences in steroid metabolism between the acne and control groups but skin from acne patients did form rather more 5α-dihydrotestosterone from testosterone than did skin from control subjects. Skin from the shoulder (acne area) of acne and control subjects metabolized dehydroepiandrosterone more rapidly than did skin from the thigh (non-acne area); this appeared to be due to higher activity of 3β-hydroxysteroid dehydrogenase Δ^{4-5} isomerase in shoulder than thigh skin.

A comparison of steroid metabolism in skin incubated in Krebs Ringer medium with that in cryostat sections of skin incubated in a tris-buffered medium showed that the method of incubation could affect the quantitative pattern of steroid metabolism *in vitro*.

Although various androgens, e.g. dehydroepiandrosterone sulphate, dehydroepiandrosterone, androstenedione and testosterone, circulate in human plasma, testosterone is often thought of as the most important androgen. However, in many androgen target organs testosterone is converted to 5α-dihydrotestosterone, and the biological action of testosterone may be mediated by the formation of 5α-dihydrotestosterone in the target organs (Bruchovsky & Wilson, 1968a, b). It is known that skin converts testosterone to 5α-dihydrotestosterone (Gomez & Hsia, 1968) and that the pilosebaceous unit is an androgen target organ (Strauss & Pochi, 1963). We have been studying the formation in skin of the active androgens testosterone and 5α-dihydrotestosterone not only from testosterone but also from dehydroepiandrosterone and androstenedione. This study was intended to throw light on the role of androgens in acne vulgaris. It has been suggested that this syndrome might involve an increased response of the skin to androgens which could be due to an increased local biosynthesis of testosterone or 5α-dihydrotestosterone (Milne, 1969). Previous work (Chakraborty *et al.*, 1970) had shown that shoulder skin (an acne area) metabolized dehydroepiandrosterone more actively than did thigh or lateral chest wall skin (non-acne areas); skin from these areas was used in the present study.

MATERIALS AND METHODS

Tissue

Skin specimens from shoulder, lateral chest wall and thigh were either biopsied or obtained at the time of thoracic surgery. Subcutaneous adipose tissue was removed before the skin specimens were used. Full thickness skin biopsies were obtained from the shoulder and thigh of four healthy male students free from acne and ranging in age from 18 to 21 years. Full thickness skin biopsies were obtained from the shoulder of one female and four male subjects with severe acne of face and back who ranged in age from 17 to 28 years. These patients were taking part in a clinical trial of the drug Trimethoprim–Sulphamethoxazole (MacDonald, MacConnell & Dunsmore, 1972). The patients had received neither drug nor placebo for 4 weeks prior to biopsy.

Radioactive steroids

7α-^{3}H testosterone (1·51 Ci/mmol), 7α-^{3}H androstenedione (5·88 Ci/mmol) and 7α-^{3}H dehydroepiandrosterone (0·5 Ci/mmol) were obtained from the Radiochemical Centre, Amersham, England. They were purified before use by thin-layer chromatography on silica gel (Merck HF 254/366) using chloroform:acetone (185:15 v/v) as solvent.

Incubations

The following incubation media were used.

Krebs–Ringer system. Tissue was incubated in 1·0 ml of Krebs Improved Ringer I medium (Krebs, 1950) with either 3·5 nmol of 7α-^{3}H testosterone (2·7 μCi) or 3·5 nmol of 7α-^{3}H androstenedione (2·61 μCi) or 3·5 nmol of 7α-^{3}H dehydroepiandrosterone (1·76 μCi). The steroids were dried from methanol solution containing 5 μl of propane-1, 2-diol before addition of Krebs medium. Incubations were with shaking under 95% O_2:5% CO_2 at 37° C for 2 h. Skin was kept on ice until incubation (within 30 min of excision). Transverse slices of skin 0·5 mm thick were cut with razor blades and added to the medium. From 40 to 60 mg skin were used in each incubation.

Tris-system. Tissue was incubated in 1·4 ml of 0·1 M tris-HCl buffer at pH 7·4 containing 0·14 μmol of NAD$^+$, 0·14 μmol of NADH, 0·13 μmol of NADP$^+$, 0·13 μmol of NADPH, and either 0·70 nmol of 7α-^{3}H testosterone (1·0 μCi) or 0·18 nmol of 7α-^{3}H androstenedione (1·0 μCi). The steroids were dried down from methanol solution containing 10 μl of dimethyl formamide before addition of tris buffer. Incubation was with shaking in air for 2 h at 37° C. The tissue was frozen at −20° C immediately it was obtained and sections, 10 μ thick, were cut in a cryostat at −20° C immediately before use. From 40 to 240 mg of skin were used in each incubation.

Extraction and purification of steroids

Steroids were extracted from the tissue and medium, then purified by paper and thin layer chromatography as previously described (Chakraborty *et al.*, 1970; Hodgins, 1971; Hay & Hodgins, 1973).

Isolation and determination of steroids

Samples were removed from each purified steroid for estimation of radioactivity and estimation of carrier recovered (Chakraborty *et al.*, 1970). A further sample was taken for determination of radiochemical purity by recrystallization to constant specific activity; 10 mg carrier were used and the crystals were weighed on a Cahn Electrobalance (Cahn Instrument Co., Paramount, California, U.S.A.). No attempt was made to separate the components of the androstenediol–androstanediol fraction chromatographically but aliquots were taken for crystallization with the following carriers: androstenediol (5-androstene-3β, 17β-diol), 3α-androstanediol (5α-androstane-3α, 17β-diol), 3β-androstanediol (5α-androstane-3β, 17β-diol).

RESULTS

The metabolic products obtained on incubating testosterone, androstenedione and dehydroepiandrosterone with human skin are shown in Tables 1–5. Total recovery of radioactivity in starting material and metabolites was always greater than 80%. Fig. 1 summarizes the pathways of C_{19}-steroid metabolism in skin. Fig. 2 compares the activity of 3β-hydroxysteroid dehydrogenase Δ^{4-5} isomerase in shoulder and thigh skin, while Fig. 3 compares the conversion of testosterone to 5α-dihydrotestosterone in shoulder skin for acne patients and control subjects. The results are expressed as percentage conversion of starting substrate in 2 h and except for the polar neutral steroid fraction are corrected for losses occurring during the isolation of the steroids and are based on constant specific activities.

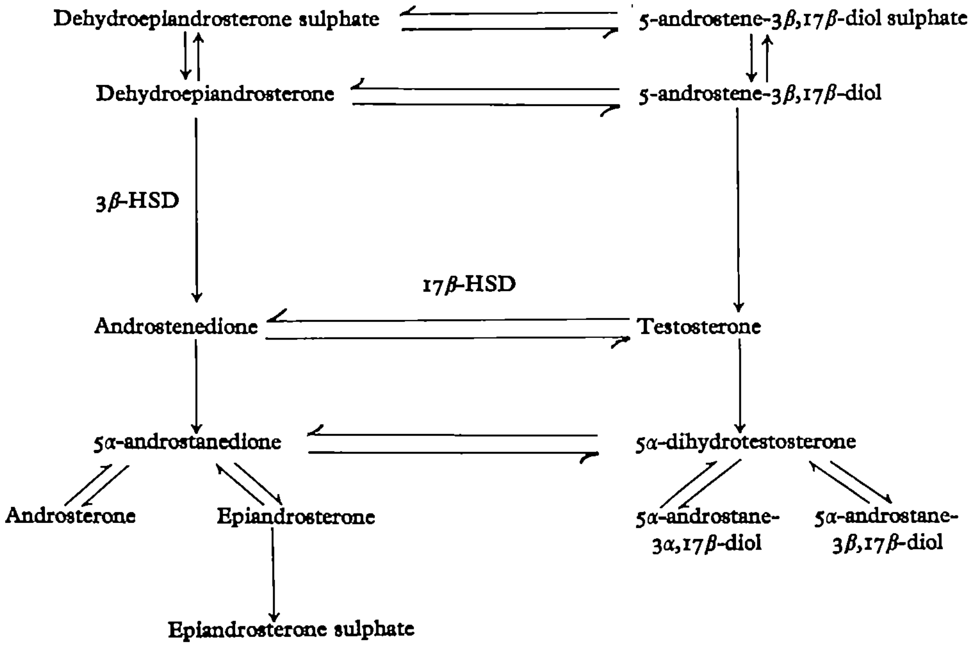

FIGURE 1. Possible pathways of C_{19} steroid metabolism in human skin. In addition to the reactions shown, dehydroepiandrosterone, androstenedione and testosterone are converted to hydroxylated metabolites. Androstenedione may be converted to 5β-androstanedione and etiocholanolone.

The products formed on incubating shoulder or thigh skin with 7α-^{3}H testosterone, 7α-^{3}H androstenedione or 7α-^{3}H dehydroepiandrosterone in Krebs Improved Ringer I medium (Krebs–Ringer system) are similar to those reported to be formed by human forehead and axillary skins (Hay & Hodgins, 1971, 1973). The main enzymes present are, as previously reported (Baillie, Calman & Milne, 1965; Faredin *et al.*, 1967; Gallegos & Berliner, 1967; Gomez & Hsia, 1968; Hodgins, 1971; Hay & Hodgins, 1971, 1973): 3β-hydroxysteroid dehydrogenase Δ^{4-5} isomerase (3β-HSD), 5α-reductase, 17β-hydroxysteroid dehydrogenase (17β-HSD), 3β-hydroxysteroid dehydrogenase and 3α-hydroxysteroid dehydrogenase. Formation of 3α-hydroxysteroids, androsterone and 3α-androstanediol, exceeded that of the 3β-hydroxysteroids, epiandrosterone and 3β-androstanediol. 17β-HSD appears to act mainly in the direction of 17-oxosteroids; androstenedione, 5α-androstanedione and androsterone are formed from testosterone but very little testosterone, 5α-dihydrotestosterone, 5α-androstanediol or androstenediol are formed from androstenedione and dehydroepiandrosterone.

Conversion of dehydroepiandrosterone to androstenedione + A-ring saturated steroids was greater

TABLE 1. Metabolites produced by shoulder skin from acne patients and control subjects and by thigh skin when incubated for 2 h with dehydroepiandrosterone

	% Conversion/50 mg tissue/2 h														
	Acne—shoulder					Normal—shoulder				Thigh					
Patient	1*	2	3	4	5	6	7	8	9	6	7	8	9	2	5
Androstenediol	0·26	0·27	0·41	0·29	0·36	0·21	0·41	0·21	0·18	0·38	0·44	0·34	0·20	0·31	0·69
5α-dihydrotestosterone	0·05	0·01	0·015	0·41	0·23	0·02	0·03	0·02	0·01	0·07	0·005	0.002	0·43	0·004	0·10
Androstenedione	1·84	0·27	0·30	0·19	0·68	0·43	0·21	0·17	0·60	0·09	0·07	0·05	0·40	0·06	0·39
5α-androstanedione	5·33	0·54	0·87	1·41	2·97	0·99	1·86	1·25	0·50	—	0·17	0·13	0·16	0·16	1·29
Androsterone	0·53	0·05	0·28	0·42	1·18	0·23	0·46	1·20	0·13	—	0·07	0·16	0·16	—	2·08
Epiandrosterone	0·70	0·09	0·12	0·14	0·27	0·03	0·24	0·13	0·004	—	0·05	0·04	0·04	0·07	0·20
Polar fraction	1·89	2·40	3·55	2·92	2·85	2·75	3·11	2·69	1·72	3·00	1·88	2·70	2·45	2·94	4·73

* Female patient.

TABLE 2. Metabolites produced by shoulder skin from acne patients and control subjects and by thigh skin when incubated for 2 h with androstenedione

	% Conversion/50 mg tissue/2 h										
	Acne–shoulder			Normal–shoulder				Thigh			
Patient	2	3	4	6	7	8	9	7	8	9	2
Testosterone	0·10	0·38	0·29	0·40	0·13	1·13	0·20	0·18	0·34	0·26	0·31
5α-dihydrotestosterone	0·09	0·16	0·17	0·12	0·06	0·004	0·11	0·08	0·11	0·07	0·21
5α-androstanedione	8·1	7·4	9·4	11·0	4·9	5·1	6·4	3·2	5·2	5·3	5·3
Androsterone	—	5·1	2·7	3·0	1·9	3·3	2·9	1·7	5·6	2·9	2·1
Epiandrosterone	0·16	0·30	0·52	0·38	0·18	0·004	0·22	0·19	0·33	0·25	0·23
Etiocholanolone	0·10	0·47	0·16	0·24	0·23	0·21	0·16	0·16	0·62	0·18	0·15
Polar fraction	0·58	0·67	1·07	0·96	0·50	0·48	0·60	0·48	0·64	0·53	0·70

in shoulder than in thigh skin; however, there appeared to be no differences in dehydroepiandrosterone metabolism between acne and non-acne groups. Similarly, there were no differences in metabolism of androstenedione between the two groups. Rather more 5α-dihydrotestosterone was formed from testosterone by shoulder skin from the acne patients than by controls, but the difference was small and fell short of statistical significance. This was, however, the only difference in steroid metabolism between the acne and control groups.

Using frozen sections of skin incubated in the tris-system, it was shown that testosterone and androstenedione were metabolized more extensively by shoulder than by lateral chest wall skin. A study of

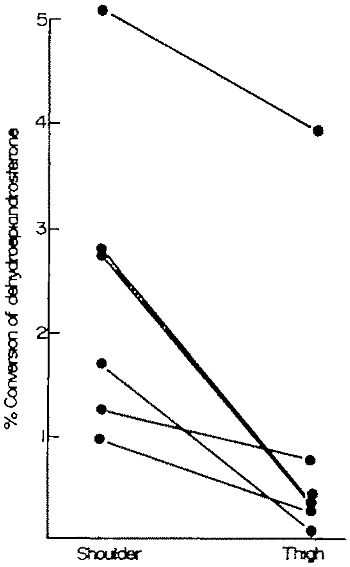

FIGURE 2. 3β-HSD activity in human skin. Skin was incubated with [7α-^{3}H] dehydroepiandrosterone as described in the text. 3β-HSD activity is expressed as the total % conversion of dehydroepiandrosterone to androstenedione + A-ring saturated steroids by 50 mg tissue in 2 h. Significance of difference $P < 0{\cdot}05$.

TABLE 3. Metabolites produced by shoulder skin from acne patients and control subjects and by thigh skin when incubated for 2 h with testosterone

	% Conversion/50 mg tissue/2 h												
	Acne—shoulder				Normal—shoulder				Thigh				
Patient	1*	2	3	4	6	7	8	9	6	7	8	9	2
5α-dihydrotestosterone	4·76	3·35	3·55	2·09	3·16	1·27	1·31	1·83	1·66	1·01	1·18	1·27	1·37
3α-androstanediol	0·69	1·05	2·28	0·63	0·72	0·85	1·17	1·28	1·42	0·19	1·46	0·87	0·84
Androstenedione	0·65	1·27	1·45	2·20	1·32	1·77	1·98	0·95	2·86	0·57	2·32	1·43	1·26
5α-androstanedione	0·91	2·30	3·43	3·16	4·96	1·25	4·64	1·03	1·22	0·43	1·36	1·49	2·05
Androsterone	—	0·49	0·90	0·78	0·93	0·47	3·31	0·25	1·03	0·17	1·27	0·74	0·72
Polar fraction	0·56	0·57	0·75	0·58	0·46	0·73	0·59	0·34	0·78	0·45	0·76	0·40	0·55

* Female patient.

TABLE 4. Metabolites produced by thin frozen sections of skin when incubated for 2 h with testosterone in the tris-system

	% Conversion/100 mg tissue/2 h										
Patient	Male 37 years		Male 67 years		Male* 26 years		Female† 31 years		Female‡ 21 years		Female 18 years
Body site	Shoulder	LCW§	Shoulder	LCW	Shoulder	Thigh	Shoulder	Thigh	Shoulder	Thigh	Shoulder
5α-dihydrotestosterone	0·96	—	0·79	1·4	0·56	0·58	2·0	1·6	1·0	0·08	2·6
3β-androstanediol	—	—	0·16	—	0·58	—	1·2	0·77	0·84	0·08	0·65
Androstenedione	3·3	0·54	23·9	1·4	17·0	3·5	16·8	3·0	7·0	0·96	8·9
5α-androstanedione	—	—	—	—	—	—	1·8	0·36	0·4	—	0·96
Androsterone	—	—	—	—	0·03	—	—	—	—	0·04	—
Epiandrosterone	0·37	—	0·48	—	—	—	0·17	0·04	—	—	—

* Patient with severe seborrhoea.
† Hirsutism on legs.
‡ Hirsutism confined to face.
§ LCW, lateral chest wall.

TABLE 5. Metabolites produced by thin frozen sections of skin when incubated for 2 h with androstenedione in the tris-system

	% Conversion/100 mg tissue/2 h						
Patient	Male 67 years		Male 46 years	Male 59 years	Female 32 years	Female 18 years	
Body site	Shoulder	LCW*	Shoulder	Shoulder	Shoulder	Shoulder	LCW
Testosterone	2·3	1·6	3·3	3·4	4·7	5·3	1·3
5α-dihydrotestosterone	0·32	—	—	0·66	0·18	—	—
3β-androstanediol	—	—	—	—	—	0·27	—
5α-androstanedione	10·7	2·4	1·6	0·56	1·3	6·9	2·4
Androsterone	0·31	0·07	—	—	—	0·54	0·07
Epiandrosterone	1·9	1·5	4·8	2·9	6·2	9·7	1·5

* LCW, lateral chest wall.

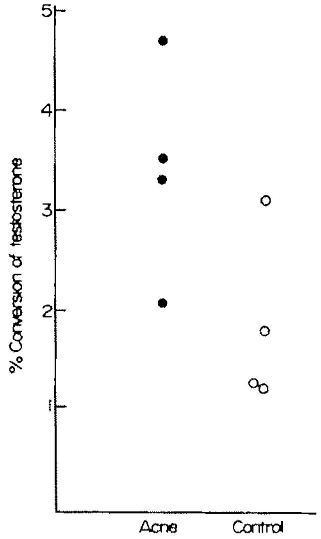

FIGURE 3. Conversion of testosterone to 5α-dihydrotestosterone in shoulder skin. Shoulder skin from individuals with acne and from control subjects was incubated with [7α-^{3}H] testosterone as described in the text. The % conversion of testosterone to 5α-dihydrotestosterone by 50 mg tissue in 2 h is shown. Significance of difference $0{\cdot}05 < P < 0{\cdot}1$.

testosterone metabolism in shoulder and thigh skin of two females with idiopathic hirsutism showed that thigh skin from one patient, with hirsutism confined to the face, formed much less 5α-dihydrotestosterone than did her shoulder skin; while the thigh skin from the other patient, with extensive growth of coarse hair on this site, produced almost as much 5α-dihydrotestosterone as did her shoulder skin.

There were some quantitative differences in the pattern of metabolites formed by shoulder skin incubated in the Krebs–Ringer or tris-system. In the Krebs–Ringer system the formation of the 3α-hydroxylated metabolites, androsterone and 3α-androstanediol, predominated over the formation of the 3β-hydroxylated metabolites, epiandrosterone and 3β-androstanediol; in the tris-system the reverse situation was found.

DISCUSSION

It is thought that in many target organs 5α-dihydrotestosterone is the biologically active metabolite of testosterone (Bruchovsky & Wilson, 1968a, b). The conversion of testosterone to this metabolite in human skin suggests that 5α-dihydrotestosterone may be the biologically active androgen in this organ. Increased production of 5α-dihydrotestosterone from other androgenic steroids circulating in the plasma could be a local source of excess androgen in skin which might lead to hyperactivity of the sebaceous glands and eventually to acne. Another possibility is that decreased catabolism of 5α-dihydrotestosterone to 17-oxosteroids or androstanediols, perhaps due to binding to specific tissue proteins (Mainwaring, 1969), could lead to excessive androgenic stimulation of sebaceous gland secretion.

The finding that dehydroepiandrosterone and androstenedione were only converted to 5α-dihydrotestosterone to a small extent in shoulder and thigh skin is in accord with the finding of Pochi & Strauss (1969) that these two steroids are considerably less active than testosterone in stimulating sebum production in men.

In the acne study, four persons were controls and five were acne patients. The earlier studies of Baillie *et al.* (1965) and Chakraborty *et al.* (1970) indicated that the potential conversion of dehydroepiandrosterone to more active androgens was greater in skin taken from acne prone areas than from non-acne areas. The present finding of greater conversion of dehydroepiandrosterone to androstenedione + A-ring saturated steroids in shoulder than thigh skin confirms this. However, the lack of any differences in dehydroepiandrosterone or androstenedione metabolism between the acne and non-acne groups tends to suggest that specific changes in 3β-HSD or 17β-HSD activity are not directly involved in acne in the male.

Sansone & Reisner (1971) examined the metabolism of testosterone in skin and found that acne sufferers formed more 5α-dihydrotestosterone than did controls the differences being more pronounced for women than men; other metabolites of testosterone were not studied in detail. The finding of rather more conversion of testosterone to 5α-dihydrotestosterone by the acne group than by controls in the present study is in agreement with the work of Sansone & Reisner, but the difference was much less distinct. Some androgen metabolizing enzymes may be localized mainly in sebaceous glands (Baillie *et al.*, 1965), the growth of which is androgen controlled and age dependent (Strauss & Pochi, 1963, 1965). While our acne patients and controls were of similar ages, it is not clear from the work of Sansone & Reisner (1971) whether the increased conversion of testosterone to 5α-dihydrotestosterone observed in their acne group was the sole metabolic change or merely reflected differences in the overall rate of steroid metabolism due to comparison of acne lesions of young patients, having more active sebaceous glands, with skin from normal controls of unspecified ages. A possible example of this problem was encountered when steroid metabolism in forehead skin from a man aged 20 years, free from acne, was compared with that in forehead skin of two men aged 58 and 83 years (Hay & Hodgins, 1973). Metabolism of testosterone in the skin from the young man was more extensive than in the older specimens; while the formation of 5α-dihydrotestosterone was much greater, the conversion of testosterone to other metabolites was also higher. Such an age difference in skin steroid metabolism would be in agreement with the observation that the rate of clearance of testosterone from the blood decreases with advancing age in men (Vermeulen, Rubens & Verdonck, 1972). In our present study, 5α-dihydrotestosterone was the only metabolite of testosterone which appeared to be formed in greater amounts in acne than non-acne shoulder skin.

Regarding body site differences in skin steroid metabolism, the 3β-HSD appeared to be the only enzyme whose activity was consistently greater in shoulder than in thigh skin (Fig. 2), although there was slightly more conversion of testosterone to androstenedione + 5α-androstanedione in three of the shoulder skins than in the corresponding thigh skins (Table 3). Chakraborty *et al.* (1970) suggested that the higher 3β-HSD activity in shoulder skin was due to larger and more numerous sebaceous glands in this than in thigh or lateral chest wall skin, as histochemical studies had localized the enzyme in sebaceous glands (Baillie *et al.*, 1965). Histological examination of the skin slices used in the present study showed the shoulder skins to have large sebaceous glands while the thigh specimens had only tiny glands associated with large terminal hairs. A concentration of 3β-HSD in the sebaceous glands would therefore correlate with the shoulder–thigh difference in conversion of dehydroepiandrosterone to androstenedione. It has been reported that 5α-reductase and 17β-HSD activities are relatively high in plucked hair follicles (Northcutt, Island & Liddle, 1969; Fazekas & Lanthier, 1971), which may explain in part the extent of testosterone and androstenedione metabolism in male thigh skin.

The studies with the tris-system, which is similar to that used by Chakraborty *et al.* (1970), showed that testosterone and androstenedione were more actively metabolized in the shoulder than in lateral chest wall skin of males and females and that 17β-HSD activity in particular was higher in the shoulder than in the thigh skin of two females with hirsutism. This latter body site difference was in contrast to the results obtained with the shoulder and thigh skin of the young male acne patients and controls. The extent of conversion of testosterone to 5α-dihydrotestosterone by the thigh skin of the female with hirsutism affecting this site was almost as high as that by her shoulder skin; this was like the results obtained when skin from young men was studied using the Krebs–Ringer system. However, the female whose hirsutism was confined to the face formed very little 5α-dihydrotestosterone in her thigh skin. This indication of possible metabolic differences between male and female thigh skin and of a shift in testosterone metabolism towards the male pattern in the hirsute female thigh skin suggests that a change in skin steroid metabolism might be involved in idiopathic hirsutism as well as in acne vulgaris.

The quantitative differences in metabolite patterns formed by shoulder skin incubated in the Krebs–Ringer or tris-systems are considered here as they bear both on the validity of comparing the results obtained by different workers using different incubation systems and on extrapolating from the *in-vitro* to the *in-vivo* situation. All our other studies of human skin from various body sites of individuals ranging in age from 18 to 83 years have shown far greater formation of 3α- than 3β-hydroxysteroids in the Krebs–Ringer system (Hodgins, 1971; Hay & Hodgins, 1973, and unpublished work). In the present study of the two incubation systems tissue samples can be compared from the same body site; the age ranges of the skin donors are rather different but they overlap and it seems likely that the different extent of formation of 3α- and 3β-hydroxysteroids must be due to the incubation systems. Preliminary experiments have indicated that freezing and thawing of the tissue during freeze-sectioning of the skin for tris-incubations may selectively inhibit some enzyme activities, particularly the 3α-hydroxysteroid dehydrogenase.

Our previous studies showed that human skin could form 3β-androstanediol from testosterone, androstenedione and dehydroepiandrosterone (Hay & Hodgins, 1971, 1973). This metabolite was not detected in the present study when shoulder or thigh skin were incubated in the Krebs–Ringer system, but the studies with the tris-system showed that skin from these sites is at least potentially able to form 3β-androstanediol. There is some evidence that, like 5α-dihydrotestosterone, this steroid may be an active metabolite of testosterone (Robel, Lasnitizki & Baulieu, 1971), and it is known to stimulate sebum secretion in the rat (Nikkari & Vakavaara, 1970). Thus studies of the factors determining the relative conversion of testosterone to this 3β-hydroxy metabolite and the corresponding 3α-hydroxy isomers in skin might shed light on the mechanisms controlling sebaceous gland growth.

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Plasma androgens in acne vulgaris

L.S.LIM AND V.H.T.JAMES*

Departments of Dermatology and Chemical Pathology,
St Mary's Hospital Medical School, London W2 1PG

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SUMMARY

In a group of patients with acne, the levels of plasma 17β-hydroxysteroids (a group of naturally occurring androgens), their diurnal variation and their binding to plasma proteins were investigated. No clear abnormality of plasma 17β-hydroxysteroid levels was present in the patients and neither was any consistent pattern of change seen through the 24 h.

Basal (10·00 h) levels of total plasma 17β-hydroxysteroids were found to be elevated in four out of eighteen female patients with acne. These women were clinically indistinguishable from other female patients.

The mean level of free 17β-hydroxysteroids was significantly higher in the female patients with acne than in controls ($P<0{\cdot}01$). However, in male patients neither the mean basal level of plasma 17β-hydroxysteroids nor that of the free fraction was significantly different from controls.

Indirect estimation of testosterone-binding globulin concentration also showed no differences either in the range, or in the mean level between patients and controls of both sexes.

It is possible that the elevated level of free 17β-hydroxysteroids found in a few female patients contributed to the pathology of their disorder.

The demonstration that testosterone and dihydrotestosterone increase the size and secretory activity of sebaceous glands in man (Strauss, 1962) suggested that androgens may be implicated in the pathogenesis of acne vulgaris. Various abnormalities of androgen metabolism might be present, such as increased levels of circulating androgens, variation in the binding of androgens to testosterone-binding globulin (TeBG), or the skin itself might synthesize excess androgen or possess increased end-organ sensitivity.

In a previous comparison of random total plasma testosterone levels between acne and normal male subjects no difference was noted between the two groups (Pochi *et al.*, 1965). It is now known that there is a diurnal variation of plasma testosterone level in normal subjects (Boon, Keenan & Slaunwhite, 1972) and the possibility arises that there may be a different pattern of diurnal secretion of androgens in acne subjects. Amongst the most potent natural androgens are those steroids with an hydroxyl group in the 17β- position of the molecule (Murphy, 1970), and their assay as a group, rather than estimation of testosterone, might more readily reveal any difference between acne and normal subjects. The aim of this study was to investigate in patients with acne the circulating levels of 17β-hydroxysteroids, their diurnal variation and their relationship to the diurnal variation of plasma cortisol. In addition, the binding of these androgens to plasma proteins was studied.

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* Reprint requests to Professor V.H.T. James.

PATIENTS AND METHODS

Diurnal studies

Three male patients aged 15, 21 and 22 years with acne and two normal males aged 22 and 24 years were admitted to hospital for half-hourly blood sampling over a 24 h period, and during this time they were confined to bed. Heparinized blood specimens were centrifuged, and the plasma frozen at −20°C and stored in plastic vials until required for assay.

Basal 10.00 h 17β-hydroxysteroid levels and protein binding

Plasma was obtained from the following groups of patients: eighteen normal females aged 15–35 years; twenty-six female patients with acne aged 14–35 years; eleven normal males aged 21–33 years; and eleven male patients with acne aged 15–23 years.

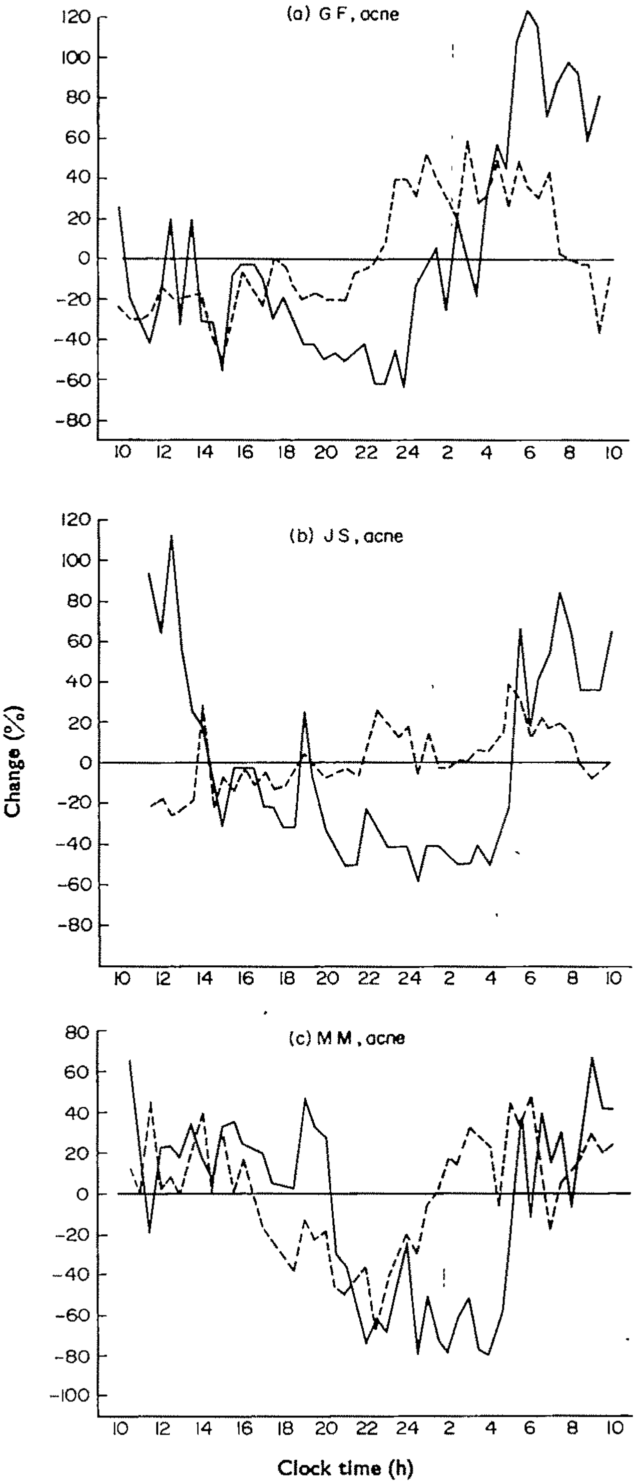
Plasma 17β-hydroxysteroid levels were measured by a competitive protein binding technique (James & Rippon, 1973) based on competition between labelled testosterone and the unlabelled plasma androgens for the available sites of testosterone-binding globulin (TeBG). The latter was obtained from third trimester pregnancy plasma. The 17β-hydroxysteroids were extracted from plasma with a hexane/ether mixture, and following evaporation to dryness were incubated with TeBG and tritiated testosterone of known radio-activity. The displaced and therefore unbound labelled testosterone was separated by ammonium sulphate precipitation of the TeBG. After centrifugation, radioactivity was measured in a Packard liquid scintillation counter. When standard amounts of unlabelled testosterone were similarly processed, a graph could be plotted in which the percentage free radio-active testosterone was a function of the unlabelled testosterone, and in this way the mass of 17β-hydroxysteroid in each test sample could be read from the graph and expressed as ng/100 ml. The precision for this method is 12% for inter-assay and 7% for intra-assay measurements.

Plasma cortisol was also measured by competitive protein binding (Murphy, 1967). Testosterone-binding globulin was estimated by the method of Rosner (1972), the result being expressed as μg of dihydrotestosterone bound by 100 ml of plasma. The percentage binding of plasma testosterone to the testosterone-binding globulin was measured by the equilibrium dialysis method of Forest, Rivarola & Migeon (1968), so that the free fraction could be calculated knowing the total plasma level and the percentage binding.

RESULTS

Diurnal studies

Fig. 1 shows the results of the diurnal studies. Steroid levels have been calculated as a percentage change from the mean level over a 24 h period. The diurnal pattern for plasma 17β-hydroxysteroids appeared to be different for each of the three acne subjects investigated. One subject (M.M., Fig. 1c) showed a distinct diurnal change, whilst in a second subject (C.F., Fig. 1a) the level of plasma 17β-hydroxysteroids remained fairly constant throughout most of the day until 22.00 h when a rise occurred with nocturnal spikes, the highest being reached between 01.00 h and 07.00 h. This pattern is also seen in one of the control subjects (G.J., Fig. 1d) in whom the rise of 17β-hydroxysteroids also commenced at 22.00 h and peak levels were reached between 02.00 h and 07.00 h. In the remaining patients with acne (J.S., Fig. 1c) and control subject (C.C., Fig. 1e) no distinct diurnal change was


(a) G F, acne
120
100
80
60
40
20
0
-20
-40
-60
-80
10 12 14 16 18 20 22 24 2 4 6 8 10
(b) J S, acne
120
100
80
60
40
20
0
-20
-40
-60
-80
10 12 14 16 18 20 22 24 2 4 6 8 10
(c) M M, acne
80
60
40
20
0
-20
-40
-60
-80
-100
10 12 14 16 18 20 22 24 2 4 6 8 10
Change (%)
Clock time (h)

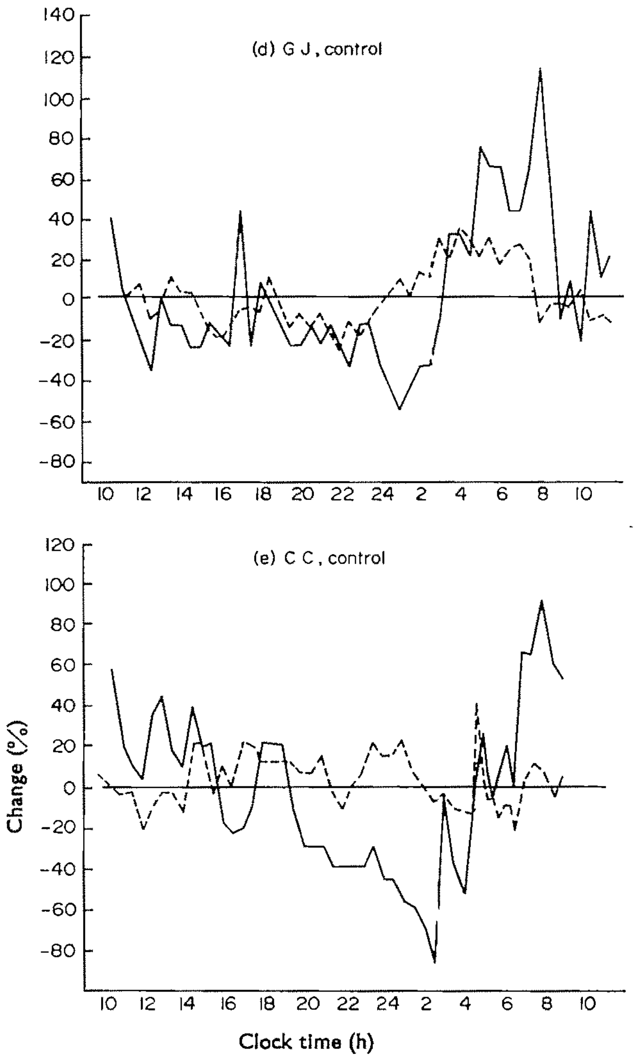


FIGURE 1. Diurnal variation of plasma 17β-hydroxysteroids and plasma cortisol. Results are expressed as percentage change from the mean over the 24 h period. Ordinate, percentage change; abscissa, clock time. —, plasma cortisol; - - - -, plasma 17β-hydroxysteroids.

apparent. The levels of plasma 17β-hydroxysteroids appeared to fluctuate around the mean with no distinct times for maximum or minimum levels.

Table 1 gives the range and mean level for each subject. The mean levels of plasma 17β-hydroxysteroids in all five subjects fall within the range for normals and the lowest level occurred in M.M., the youngest subject investigated, who was a 15-year-old boy with acne.

Fig. 1 shows the changes in the levels of plasma cortisol, and plasma 17β-hydroxysteroids throughout the 24 h. There was no consistent correlation, although in subjects G.F., M.M. and G.J., the general pattern of change was similar. In all three subjects, the nocturnal rise in cortisol occurred some time later than the rise in 17β-hydroxysteroids. All subjects showed a distinct rhythm of plasma cortisol levels with an identical range (Table 2).

Basal plasma 17β*-hydroxysteroids*

Fig. 2 shows the range for basal (10·00 h) plasma 17β-hydroxysteroids level in acne and control subjects of both sexes. Four women with acne had a high level of basal plasma 17β-hydroxysteroids. In the remainder the levels were similar to those found in controls. The difference in the mean level for the acne and control groups is not statistically significant ($P > 0{\cdot}1$). In males there is also no significant difference in the range or in the mean for the two groups ($P = 0{\cdot}3$).

TABLE 1. Range and mean levels of plasma 17β-hydroxysteroids through 24 h in three patients with acne and two normal subjects

	Subject	Age (years)	Range (ng/100 ml)	Mean
Acne	G.F.	21	325–1040	640
	J.S.	22	440– 825	598
	M.M.	15	133– 660	490
Normal	G.J.	22	750–1340	1013
	C.C.	24	520–1020	694

TABLE 2. Range and mean levels of plasma cortisol through 24 h in three patients with acne and two normal subjects

	Subject	Age (years)	Range (μg/100 ml)	Mean
Acne	G.F.	21	3 –21	8·8
	J.S.	22	4 –22	10·3
	M.M.	15	2·4– 19·2	10·9
Normal	G.J.	22	2·5–19·5	9·4
	C.C.	24	4 –19	9·2

Dihydrotestosterone binding capacity

Fig. 3 shows the results for the estimation of plasma TeBG, which is expressed as the mass of radioactive dihydrotestosterone bound by 100 ml of plasma. There was no significant difference between acne and control subjects. One female subject with acne had a slightly lower level of TeBG than the lowest found in normals.

Free plasma 17β*-hydroxysteroids*

In women, levels of free plasma 17β-hydroxysteroids were significantly higher in acne than in controls (Fig. 4, $P < 0{\cdot}01$), but in males this difference was not significant.

DISCUSSION

In this study the possibility that subjects with acne may have abnormal levels of plasma androgens was investigated. It was decided to measure plasma '17-hydroxysteroids' on the basis that this might more readily reveal any abnormality in total androgen level than would the more specific estimation of plasma testosterone. In addition, since the pattern of androgen levels throughout the 24 h is not yet well established, it was felt desirable to try to study this as well. It is possible that

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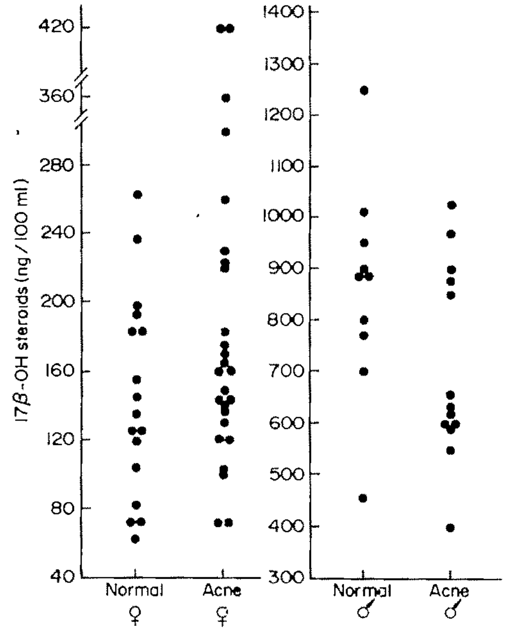


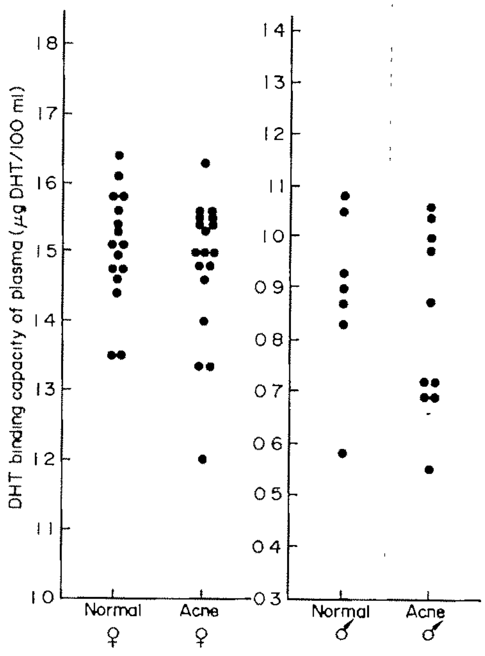
	Normal	Acne
Women		
Mean ± SD (ng/100 ml)	144 ± 58	190 ± 107 ($P > 0{\cdot}1$)
Range	62–266	73–420
Men		
Mean ± SD (ng/100 ml)	808 ± 207	698 ± 207 ($P = 0{\cdot}3$)
Range	450–1050	400–1050

FIGURE 2. Plasma 17β-hydroxysteroid levels in subjects with acne and in controls.

single plasma readings may be unrepresentative of the overall picture if fluctuations of plasma levels occur through the day.

Investigations of 17β-hydroxysteroid levels throughout the 24 h did indeed reveal a fluctuating pattern, both in the patients with acne and in control subjects. However, no clear abnormality was seen in the patients neither was any consistent pattern of change seen throughout the 24 h. In some subjects, a fairly marked diurnal change was seen. There was no clear cut correlation though, with plasma cortisol levels, and it therefore seems unlikely that changes in ACTH secretion are the major cause of the fluctuations in 17β-hydroxysteroid levels. Since the major contributor to the plasma 17β-hydroxysteroids is testosterone, together with smaller amounts of dihydrotestosterone, 5α-androstane-3α, 17β-diol and 5-androstene-3β, 17β-diol, it would be expected that in male subjects the gonadal secretion of testosterone would be by far the most important factor determining the absolute level of 17β-hydroxysteroids, although superimposed on this, there may be additional amounts which are adrenal in origin.

The range for basal levels of plasma 17β-hydroxysteroids, either as the free fraction or as the total plasma level, was similar for male patients with acne and control subjects. Pochi *et al.* (1965) found a



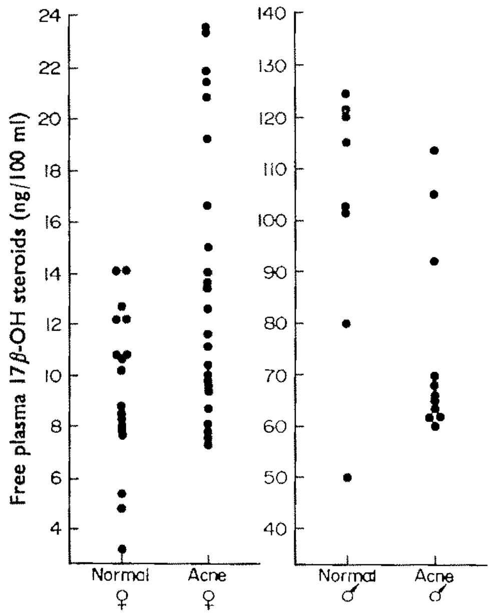
	Normal	Acne
Women		
Mean ± SD (ng DHT/100 ml)	1·49±0·13	1·49±0·1
Range	1·35–1·64	1·2–1·63
Men		
Mean ± SD (ng DHT/100 ml)	0·89±0·17	0·87±0·2
Range	0·58–1·08	0·55–1·16

FIGURE 3. Dihydrotestosterone (DHT) binding capacity of plasma in subjects with acne and in control subjects.

similar result in their study of plasma and urinary testosterone. In the majority of females with acne, basal plasma 17β-hydroxysteroids levels fell within the range of that for controls, but in four of them it was elevated. The difference in the mean for the two groups was not statistically significant. These few women with elevated 17β-hydroxysteroids were clinically normal apart from having acne, and showed no signs of virilism. Their ages were 18, 20, 21 and 35 years and they were not taking contraceptive drugs. Plasma testosterone in one of them was elevated (>128 ng/100 ml), whilst in two others it was in the upper range of normal (69 and 64 ng/100 ml; normal range 15–65).

The free 17β-hydroxysteroids were elevated in seven women with acne but in the majority they were within the range for normals. The difference in the mean level of free 17β-hydroxysteroids for the two groups is statistically significant.

The dihydrotestosterone binding capacity of plasma gives an estimate of the amount of TeBG that is present. The free fraction of 17β-hydroxysteroids is inversely related to TeBG level for a given



	Normal	Acne
Women		
Mean ± SD (ng/100 ml)	9·5±3·0	13·65±5·3 ($P<0·01$)
Range	3·6–14·1	7·3–23·8
Men		
Mean ± SD (ng/100 ml)	103±27·8	76·8±18 3
Range	50–129	60–115

FIGURE 4. Levels of free plasma 17β-hydroxysteroids in subjects with acne and in controls.

plasma level. Only one female acne subject had low dihydrotestosterone binding capacity, and in all the others this was similar to controls.

This study demonstrates that in the majority of patients with acne, abnormality of the plasma androgen levels seems unlikely to be a major causative factor, although this may be the case in a few women who have elevated levels of free circulating androgens.

Vermeulen, Rubens & Verdonck (1972) found a significant correlation between the excretion of urinary androstanediol, a metabolite of testosterone, and the free testosterone fraction in plasma. Urinary androstanediol excretion reflects to some extent extra-hepatic metabolism of testosterone. Mauvais-Jarvis, Charransol & Bobas-Masson (1973) have measured the 24 h urinary excretion of androstanediol and testosterone and found a marked increase in androstanediol excretion in women with hirsutism, and to a lesser extent in women with acne. Testosterone excretion in these subjects was only slightly increased or comparable to normal. The inference drawn from this study was that extra-hepatic conversion of testosterone to dihydrotestosterone and thence to androstanediol is greater in women with hirsutism or acne than it is in normals. Their data would seem to correlate well with the findings of Sansone & Reisner (1971) that skin from males and females with acne can convert testosterone to dihydrotestosterone at a much higher rate than their normal counterparts.

The conclusion from this study is therefore that in patients with acne, it has not been possible with the methods employed to demonstrate any consistent abnormality of androgen metabolism. However, in some patients, who could not be distinguished clinically from the others, it was possible to show increased plasma levels of free testosterone as compared with control subjects and it is attractive to suggest that this is related to the presence of acne. The problem of why only some of the patients studied show this abnormality, or how it originates, requires further study.

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HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies

A. SVEJGAARD,* L. STAUB NIELSEN,* E. SVEJGAARD,†
F. KISSMEYER NIELSEN,‡ A. HJORTSHØJ§ AND H. ZACHARIAE§

* Tissue-Typing Laboratory of the Blood-Grouping Department and † Department of Dermatology, Rigshospital, University of Copenhagen, and ‡ Tissue-Typing Laboratory, Aarhus Kommunehospital, and § Department of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark

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SUMMARY

A collaborative study of HL-A typing was done on 156 unrelated patients with psoriasis vulgaris and thirty-one patients with pustular psoriasis. Highly significant increases of HL-A13 and HL-A17 were found in psoriasis vulgaris: about half of the patients carried either of these antigens as compared to about 12% of the controls. The effect of HL-A13 and 17 is confined almost exclusively to psoriasis with onset before the age of 35 years. In contrast, none of these antigens was increased in pustular psoriasis, indicating that pustular and common psoriasis are different aetiological entities. Family studies in psoriasis vulgaris showed that psoriasis can develop in relatives lacking the HL-A haplotype following psoriasis in the other family members. Hence, it seems most likely that HL-A represents only part of the genetic basis of psoriasis vulgaris. This disease is probably of a polygenic threshold character, and HL-A13 and 17 decreases the threshold making the expression of psoriasis more likely in individuals carrying these determinants in addition to other probably non-HL-A linked psoriasis determinants. This concept is supported by the observation of a higher incidence of a positive family history in HL-A13 and/or 17 positive patients than in patients lacking these antigens. Studies of antistreptolysin O and antistreptococcal hyaluronidase titres and of triggering streptococcal infections yielded no clue as to the pathogenetic effect of HL-A. The frequencies of HL-A13 and 17 were not significantly increased in twenty-one patients with psoriatic arthritis, but HL-A27 may be increased in these patients. This study shows the importance of HL-A in the subdivision of diseases and stresses the importance of family studies for clarifying the associations between HL-A and disease.

Among the associations between various diseases and antigens of the human major histocompatibility system, HL-A, the one between psoriasis and HL-A13 and 17 is one of the most well established (Russel, Schultes & Kuban, 1972; White *et al.*, 1972). The frequencies of both these antigens in psoriatics are increased by about three times. Furthermore, in one large kindred studied, it was found that psoriasis was only present in those family members who carried the HL-A1, 17 haplotype (Russel *et al.*, 1972). In order to study this relationship further, we undertook a study of psoriatics

* Reprint requests to Dr A. Svejgaard.

and their relatives concentrating also on the distinction between the two major forms of psoriasis, psoriasis vulgaris and pustular psoriasis.

MATERIALS AND METHODS

Patients

A total of 187 unrelated Caucasian psoriatics were included in the study. The diagnosis and origin of the patients are given in Table 1. We have not distinguished between localized and generalized pustular psoriasis, but recognized chronic palmoplantar pustulosis as a type of its own. Among our patients classified within this group none had ordinary psoriasis or pustular lesions localized to parts of the skin other than palms and soles. Patients with pustular bacterid, a debatable part of the psoriatic spectrum, may have been included in this group. In addition, we studied eight families with more than one case of psoriasis vulgaris. All patients and relatives were seen by specialists in dermatology and had a definite diagnosis of psoriasis. Most of them have been hospitalized for this condition on one or more occasions. The diagnosis of psoriatic arthritis was based on obvious arthritic abnormalities of the distal interphalangeal and/or sacroiliac joints by X-ray examination. All patients classified as arthritic also had clinical signs of arthropathy on physical examination, most had increased red blood cell sedimentation rate, and all had negative serological tests for rheumatoid arthritis.

TABLE 1. Unrelated psoriatic patients

Diagnosis	No. of patients Copenhagen	Aarhus	Total
Psoriasis vulgaris	80	76	156
Pustulosis			
Generalized and localized pustular psoriasis	1	13	14
Chronic palmoplantar	3	14	17
Total	84	103	187

Controls

These consisted of 1541 healthy unrelated individuals from Copenhagen and 562 blood donors from Aarhus. The LA and FOUR phenotype distribution of these individuals did not differ significantly from Hardy-Weinberg expectations, and there were only slight differences between the antigen frequencies of the two control populations.

HL-A typing

HL-A typing was performed on freshly drawn blood samples by the method of Kissmeyer-Nielsen & Kjerbye (1967). Although the same sera were not used for all specificities, the quality of the typing in Copenhagen and Aarhus has been established by extensive exchange of cells through the co-operation of Scandiatransplant. All individuals were investigated for the antigens HL-A1, 2, 3, 9, 10, 11, W28, W19 of the LA series and HL-A5, 7, 8, 12, 13, 14, 17, 27, W5, W10, W15, W16, W18, W21 and W22 of the FOUR series.

Antistreptolysin O (ASO) and anti-streptococcal hyaluronidase (ASH) serum titres were performed at the Danish State Serum Institute under the supervision of Dr E.Kjems.

Calculations

The relative risks of Woolf (1955) were used for estimating deviations of antigen frequencies, for significance testing and for combining the results from various materials. The relative risk is the ratio hp (1–hc)/hc(1–hp), where hp and hc are the frequencies of the antigen in patients and controls, respectively. The relative risk is identical to the cross-product ratio in the 2×2 table, and the chi squares obtained are equivalent to those obtained in the ordinary chi square test without Yates' correction. In addition, ordinary chi square tests with Yates' correction, Fisher's exact test, and rank–sum testing were used. Haplotype frequencies and delta values in unrelated individuals were estimated by the method of Mattiuz *et al.* (1970).

RESULTS

Association between HL-A and psoriasis

Table 2 shows the frequencies and relative risks of the various HL-A antigens in 156 patients with psoriasis vulgaris as compared to the controls. There was probably significant ($P<0{\cdot}05$) heterogeneity between the relative risks in the comparisons only for the HL-A27 antigen and this could be due to chance. The estimates of relative risks differ significantly ($P<0{\cdot}01$) from one for the antigens HL-A7, 8, 13 and 17. The chi squares for the increased frequencies of the latter two antigens are extraordinarily high, and these deviations cannot be due to chance even considering the large number (twenty-three) of comparisons made. Only one of the patients carried both HL-A13 and 17. In our registry of HL-A typed individuals we found four carrying both HL-A13 and 17 and none of them had psoriasis.

It is evident from Table 2 that the frequencies of HL-A13 and 17 are not increased in pustular psoriasis. For both of these antigens there are significant differences ($P<0{\cdot}025$ and $P<0{\cdot}005$, respectively) between common and pustular psoriasis. The two subgroups of pustular psoriasis have been pooled as they were otherwise too small for comparison.

In Fig. 1 we have shown the 95% confidence limits of relative risks differing significantly from one when combining our data of psoriasis vulgaris with those of Russel *et al.* (1972) and of White *et al.* (1972). This was done under the assumption that there were few patients with pustular psoriasis in their material. The increased relative risks of HL-A13 and 17 are outstanding and the decreased risks of HL-A7, 8 and 12 are most likely secondary to the increases of 13 and 17. The deviations of the LA antigen frequencies are less marked than those of the FOUR antigens.

Age-at-onset

Fig. 2b and c shows the age-at-onset curves for pustular psoriasis and for psoriasis vulgaris in patients carrying HL-A13 and/or HL-A17 and in patients lacking these antigens. There is a significant difference between the two groups of psoriasis vulgaris ($P<0{\cdot}007$ using rank testing). The frequency of HL-A13 and/or 17 was significantly ($P<0{\cdot}001$) higher (59%) in patients with onset before 35 years of age than in those with later onset (23%). The increased frequency in the early age-at-onset group was found both for HL-A13 and for 17 and in both sexes. The average age-at-onset for HL-A13 and/or 17 patients was 22·3 years, while it was 28·1 years for the remaining patients with psoriasis vulgaris. Furthermore, the HL-A13–17 group has only one mode (at about 15 years), whereas there are two modes (at 15 and 55 years) for the non-HL-A13–17 group. For pustular psoriasis the curve was also bimodal with peaks at 25 and 55 years (Fig. 2a).

TABLE 2. Frequencies of and relative risks for HL-A antigens in psoriasis

Antigen	Psoriasis vulgaris							Pustular psoriasis (chronic palmoplantar pustulosis included)	
	Copenhagen		Aarhus		Relative risk	χ^2			
	Patients (n = 80)	Controls (n = 1541)	Patients (n = 76)	Controls (n = 562)		Sign.	Het.	Patients (n = 31)	χ^2
HL-A1	31	31·5	49	33·6	1·37	3·21	3·37	29	0·10
2	58	52·3	50	54·4	1·02	0·02	1·25	58	0·40
3	23	27·6	13	29·4	0·58	6·51*	2·75	32	0·28
9	15	17·3	8	15·8	0·68	2·17	1·27	16	0·04
10	11	9·3	8	7·1	1·20	0·40	0·04	3	1·29
11	14	10·0	11	13·0	1·12	0·19	1·37	6	0·54
28	6	10·3	11	8·4	0·82	0·08	1·60	10	0·00
W19	19	18·0	24	16·2	1·30	1·61	1·37	6	2·63
HL-A5	10	10 8	13	12·0	1·01	0·00	0·13	10	0·02
7	15	26·5	11	34·5	0·35	17·94‡	2·54	29	0·09
8	10	23·8	17	23·1	0·52	7·12†	1·75	23	0·01
12	18	25·7	26	21·7	0·91	0·20	3·29	39	2·72
13	15	4·2	21	2·8	5·81	48·63‡	2·74	0	1·36
14	4	4·8	4	2·1	1·16	0·12	0·98	10	1·67
17	36	8·0	28	8·2	5·50	78·67‡	1·23	3	0·95
27	14	8·1	3	8·4	1·32	0·81	5·11*	13	0·94
W5	13	13·0	14	13·0	1·04	0·02	0·12	16	0·23
W10	14	17·5	16	19·0	0·78	1·16	0·01	13	0·43
W15	10	16·9	11	19·3	0·52	5·87*	0·04	13	0·33
W16	9	5·3	9	4·6	1·90	4·50*	0·10	3	0·03
W18	10	7·3	7	6·4	1·25	0·57	0·26	3	0·78
W21	8	3·4	3	1·1	2·31	4·51*	0·00	3	0·02
W22	3	3·6	0	2·9	0·47	1·59	0·85	0	1·15

Frequencies are given as percentages. Relative risks are combined estimates for the Copenhagen and Aarhus material and are given only for psoriasis vulgaris. χ^2 sign. = chi square (1 d.f.) for the significance of the relative risks differing significantly from one. χ^2 het. = chi square (1 d.f.) for the heterogeneity between the relative risks of the two individual materials. Chi squares significant at the 5*, 1† and 0·1‡ per cent probability levels are indicated.

Psoriatic arthritis

Twenty-one patients (seventeen with psoriasis vulgaris and four with pustular psoriasis) suffered from psoriatic arthritis. Only three (18%) of the patients with psoriasis vulgaris carried HL-A13 or 17, which is significantly ($P < 0{\cdot}025$) less than the combined frequency (53%) of these antigens in the remaining patients with psoriasis vulgaris, but not significantly different from the corresponding frequency (12%) in controls. None of the pustular cases had HL-A13 or 17. Five (34%) arthritic patients with psoriasis vulgaris had HL-A27, which is significantly more frequent than the frequency of this antigen in controls ($P < 0{\cdot}005$) and in non-arthritic psoriasis vulgaris ($P < 0{\cdot}005$). Two of the five HL-A27-positive patients with arthritis had evidence of ankylosing spondylitis on X-ray investigation.

Streptococcal infections

The frequency of a history of antecedent streptococcal infections was investigated in the Copenhagen

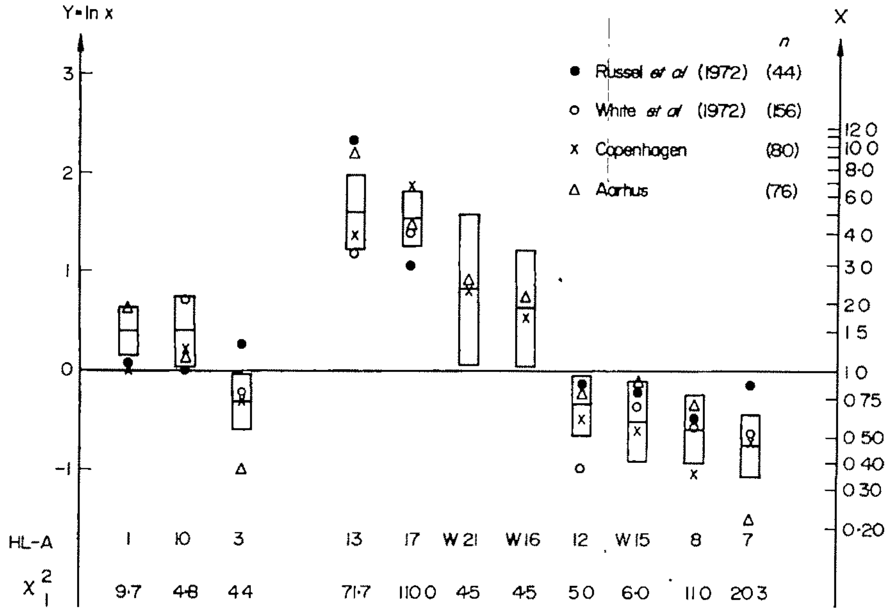

FIGURE 1. Graphical representation of relative risks (x-values) of psoriasis for various HL-A antigens in four different studies. The bars indicate 95% confidence limits of combined estimates of the relative risks. The Copenhagen and Aarhus material contained only cases with psoriasis vulgaris, while it is uncertain whether the two other studies included pustular psoriasis in addition. Relative risks are on a logarithmic scale.

material and was similar in HL-A13-positive, HL-A17-positive and HL-A13–17-negative patients with psoriasis vulgaris. ASH and ASO titres were determined in most of the 156 patients and fifty-one of these had increased titres, but the frequencies of the various HL-A antigens in this group were similar to those in the remaining patients.

Family studies

The incidence of a history of psoriasis in relatives of HL-A13 and/or 17-positive patients with psoriasis vulgaris was higher than in non-HL-A13-17 patients, who had a higher incidence than those with pustular psoriasis (Table 3).

Fig. 3 shows the segregation of psoriasis and HL-A in one of the families studied. The propositus (II,1) appears to have inherited psoriasis and the HL-A11, 17 haplotype from his mother. The mother's sister also has psoriasis but lacks the HL-A11, 17 haplotype. Accordingly, psoriasis does not travel together with the HL-A11, 17 haplotype in this family. In some of the other families studied we observed similar cases: four of thirty relatives studied had psoriasis but lacked the haplotype presumably associated with this condition in their relatives (Table 4). If psoriasis is a monogenic disease, these observations would indicate a 'recombination frequency' of 0·13 between a hypothetical psoriasis locus and HL-A. It should be stressed that our family studies are as yet too few to allow deductions as to a possible linkage between HL-A and psoriasis. However, they are sufficient to exclude close linkage.

The two segregant series of HL-A are known to be closely linked and there is association between some of the antigens from one series with some antigen of the other (Bodmer, 1973). To give an impression of the influence of this degree of linkage disequilibrium on relative risks, we estimated the relative 'risk' in normal individuals of having various LA antigens when carrying certain FOUR antigens associated with these LA antigens in the population. This was done from the phenotype

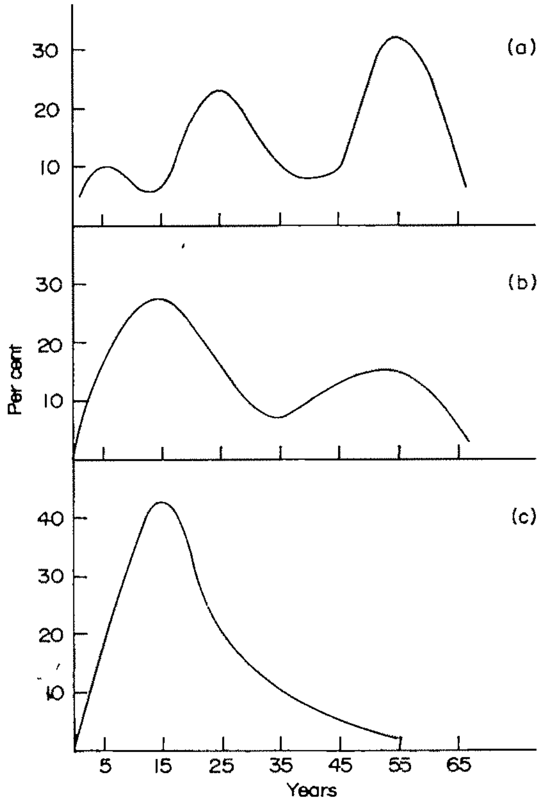

FIGURE 2. Age-at-onset curves for the three groups of patients: (a) pustular psoriasis ($n = 31$), (b) psoriasis vulgaris patients lacking HL-A13 and 17 ($n = 79$), and (c) HL-A13 and/or 17 positive patients with psoriasis vulgaris ($n = 77$). The ordinates indicate per cent patients with age-at-onset in the intervals 0–9, 10–19, 20–29, etc. years.

TABLE 3. Frequency of a positive family history

Group	Total no. of cases	Cases with family history	
		No.	Per cent
Psoriasis vulgaris*			
HL-A13	24	14	58
HL-A17	45	30	67
non-HL-A13-17	77	33	43
Pustular psoriasis	31	9	29

* The difference between the HL-A13 and/or 17 group and the non-HL-A13-17 group is significant ($\chi^2 = 5.573$, $P < 0{\cdot}025$).

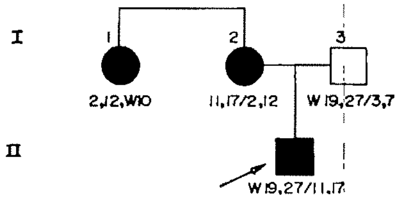

FIGURE 3. HL-A types in a kindred with psoriasis vulgaris. Solid symbols indicate psoriasis. The propositus is indicated by an arrow.

TABLE 4. Family studies in psoriasis vulgaris

Family	HL-A haplotype associated with psoriasis	No. of psoriatics in the family: The same haplotype Present	Absent	Total
A.M.N.	11,17	2	1	3
V.J.	2,17	7	0	7
B.K.	2,17	3	0	3
H.I.J.	2,17	3	1	4
M.J.	1,17	2	0	2
N.N.J.	1,17	3	0	3
E.H.	2,13	2	1	3
S.K.P.	3,7	4	1	5
Total		26	4	30

TABLE 5. Influence of linkage disequilibrium on relative risks

Antigen	Condition	Relative risk
HL-A1	HL-A8	19·81
HL-A10	W18	7·15
HL-A11	W5	4·58
W19	HL-A13	3·48
HL-A10	W16	3·30
HL-A3	HL-A7	3·27
HL-A13	Psoriasis vulgaris	5·81
HL-A17	Psoriasis vulgaris	5·50

distribution of 1541 controls, and the highest relative risks observed in this simultation is shown in Table 5. It appears that the associations between HL-A1 and 8 and between HL-A10 and W18 are the only ones which give higher relative risks than those observed for psoriasis vulgaris and HL-A13 and 17.

Estimates of haplotype frequencies from the HL-A phenotypes in 156 unrelated patients with psoriasis vulgaris revealed a general increase in the frequencies of most haplotypes carrying HL-A13 or 17 irrespective of the LA determinant present. For example, the frequencies of HL-A1, 17, HL-A2, 17, and HL-A3, 17 were increased by five, four and six times, respectively, as compared to normals. In particular, it was evident from the phenotypes that an isolated increase of the HL-A1, 17 haplotype was not involved: only twenty-one patients had both HL-A1 and 17.

DISCUSSION

Our findings clearly confirm the observation of Russel *et al.* (1972) and White *et al.* (1972) that HL-A13 and 17 occur much more frequently in psoriatics than in controls. However, our results show that this is true only in patients with psoriasis vulgaris, while there is a normal frequency of these two antigens in pustular psoriasis. Hence, the distinction between these two conditions has been substantiated by HL-A typing, supporting the view that they are distinct aetiological entities.

Like White *et al.* (1972), we found an earlier age-at-onset for HL-A13 and/or 17-positive patients with psoriasis vulgaris than in patients without these antigens. Furthermore, when studying the age-at-onset curves (Fig. 2) it appears that the effect of HL-A13 and 17 is confined mostly to early-onset psoriasis.

Two characters controlled by genes at two linked loci only very rarely give rise to association of the characters in the population. However, it appears from Table 5 that there is an association between some of the antigens from the two segregant series, LA and FOUR, of the HL-A system, although these two series are controlled by genes at two loci separated by a recombination frequency of about 1% (Svejgaard *et al.*, 1971). As pointed out by Bodmer (1973), such linkage disequilibrium may be due to selection. Nevertheless, we do not think that the high relative risks of HL-A13 and 17 in psoriasis can be explained by a similar mechanism as our family studies ruled out close linkage between HL-A and psoriasis; psoriasis occurred in several relatives lacking the HL-A haplotype common to the other psoriatic members of the family. This was true for haplotypes carrying HL-A13, HL-A17 as well as other FOUR determinants. Accordingly, it seems most reasonable to suggest that there are two or possibly more loci involved in the development of psoriasis. One of these loci is the FOUR locus or another HL-A locus closely linked to and in linkage disequilibrium with FOUR (immune recognition and/or MLC loci) and the other is/are non-HL-A linked loci, probably on other chromosomes than that carrying HL-A. This concept of psoriasis as a polygenic threshold character is supported by previous genetic studies on psoriasis (Watson *et al.*, 1971; Lomholt, 1963) and by our observation of different age-at-onset curves (Fig. 2) in patients with HL-A13 and/or 17 as compared to patients without these antigens. The presence of HL-A13 and/or 17 decreases the threshold and thus psoriasis is more likely to manifest itself in individuals having these HL-A determinants in addition to other non-HL-A linked psoriasis genes. This concept is, of course, in complete agreement with a more frequent family history of psoriasis in the HL-A13-17 group than in the remaining patients (Table 3). An early onset gives a higher chance that the disease will express itself, and thus more relatives (i.e. those carrying the relevant psoriasis genes in addition to HL-A13 or 17) are likely to be affected. We could not confirm the observation of White *et al.* (1972) that HL-A13 does not give a higher familial incidence.

The increases of HL-A13 and 17 were not confined to any particular haplotype, and accordingly, the role of LA genes seems to be small. In particular, we find no reason to assume that the HL-A1, 17 haplotype increases the susceptibility to psoriasis more than any other haplotype carrying HL-A17.

Concerning the pathogenetic function of HL-A13 and 17 (or determinants linked to these) we are left with speculations. It is a well-known observation that streptococcal infections may trigger psoriasis, particularly in young patients (Asboe-Hansen, 1971), in whom HL-A has its strongest influence. As some HL-A antigens have been reported to cross-react with streptococcal antigens (Hirata & Terasaki, 1970), it is tempting to speculate that antibodies to streptococci may cross-react with self-HL-A antigens and initiate the accelerated divisions of epidermal cells typical of psoriasis. However, the frequencies of HL-A13 or 17 were not more increased in patients with histories of triggering streptococcal infections than in patients without such a history, and furthermore, these HL-A antigens occurred with similar frequencies in patients with increased titres of AST and/or ASO as in patients with normal titres. These negative findings cannot, of course, rule out the possibility of a relationship. We have as yet been unable to find any difference between psoriasis vulgaris in HL-A13 and HL-A17-positive patients.

It is difficult to evaluate the importance of the increased frequency of HL-A27 in psoriatic arthritis. Two of our patients may have ankylosing spondylitis in which a very high frequency of HL-A27 has been reported (Caffrey & James, 1973). Moreover, this antigen is also much increased in Reiter's disease (Aho *et al.*, 1973; Zachariae, Hjortshøj & Kissmeyer-Nielsen, 1973; Friis, Svejgaard & Svejgaard, 1973), and as these three sero-negative arthropathies may often be confused a further unravelling is indicated. Perhaps, the association between HL-A27 and sero-negative arthritis is confined to patients with involvement of the sacro-iliac joint or to reactive sero-negative arthritis (Aho *et al.*, 1973).

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Plasma vitamin A levels in the common dermatoses

P.D.MIER AND JOSÉ J.M.A. VAN DEN HURK

Department of Dermatology (Professor J.W.H. Mali), University of Nijmegan, The Netherlands

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SUMMARY

The plasma vitamin A levels of 164 patients with skin disease and 102 comparable control subjects were measured by means of a modern fluorimetric technique.

No correlation was found between plasma vitamin A levels and skin disease in adults. A low or marginal vitamin A status, however, was observed in about one-third of skin patients younger than 20 years.

Vitamin A has been used as a therapeutic agent in dermatology for several decades. Although 'clinical and histological improvement sometimes occurs and may be dramatic' in the dyskeratoses (Cairns, 1972), the value of vitamin A in the common dermatoses has been the subject of considerable dispute, and such therapy has now been largely discontinued. The early literature was well reviewed by Moore (1957).

The rationale for the administration of vitamin A rests mainly on the observations that overt deficiency leads to hyperkeratotic changes and, conversely, that excess vitamin A suppresses keratin synthesis. Information regarding the actual vitamin A status of patients with skin disease, however, is scanty and conflicting. The introduction of a rapid, sensitive and specific spectrophotofluorimetric technique has therefore prompted us to carry out a survey of plasma levels in patients attending this Department.

MATERIALS AND METHODS

Patients with skin disease ('experimental group') were either attending the out-patient clinic or had been admitted for treatment to the in-patient clinic of this Department during the months June–August 1973. The control group consisted mainly of patients admitted to the Ear, Nose, and Throat Department of this hospital (Professor W.F.B.Brinkman) during the same period. Relevant data regarding the two groups are summarized in Table 1. In all cases a specimen of blood was taken between 8 a.m. and mid-day; the plasma or serum was separated and either assayed for vitamin A the same day or stored at −20°C. In the case of hospitalized patients, blood was generally taken within 48 h of admission.

Analysis was carried out by the method of Kahan (1971). 0·2 ml plasma or serum, 0·3 ml deionized water, 2·5 ml ethanol (purified by refluxing briefly with activated charcoal and filtering) and

TABLE 1. The composition of experimental and control groups according to age, sex, and clinical status. The 'miscellaneous' group consisted of photodermatoses (twelve), reticuloses (three), bullous diseases (three), condylomata (three), urticaria (two), collagen disorders (two), fungus infections (two) and individual patients with other diagnoses (seven).

Group	No.	M	F	Age (mean and range) (years)
Control subjects	102	69	33	34·1(14–72)
Experimental group (total)	164	82	82	
Psoriasis	50	35	15	38·9(14–70)
Eczema	32	17	15	37·1(17–74)
Ulcers	25	8	17	53·8(26–29)
Acne	23	9	14	20·9(15–30)
Miscellaneous	34	13	21	41·7(14–81)

2·5 ml petroleum spirit (B.P. 60–80°C, Analytic grade, Merck) were shaken vigorously for 2 min in a 10 ml glass-stoppered tube. After centrifuging to separate the phases cleanly, an aliquot of the upper layer was transferred to a 1 ml quartz cuvette. Fluorescence was measured (excitation maximum about 350 nm, emission about 470 nm) using a 'Fluorispec' spectrophotofluorimeter (SF-100, Baird Atomic) which was calibrated against a secondary standard of quinine sulphate (50 μg/litre) immediately prior to each batch of determinations. The quinine sulphate solution was subsequently calibrated against a primary standard of all-*trans*-retinyl acetate (Sigma Chemical Co.). Results were calculated as μg vitamin A alcohol per 100 ml plasma. All statistical analyses were performed using the Hewlett-Packard HP 9810 A computer.

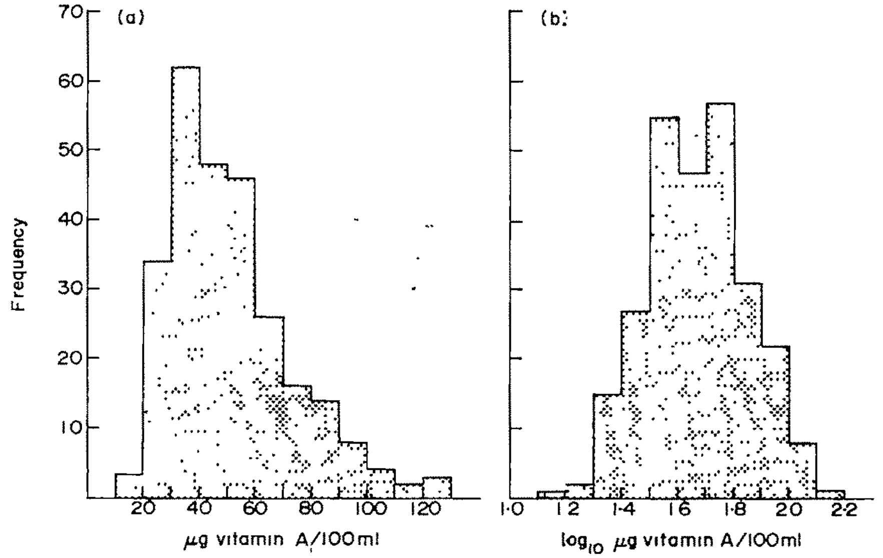

FIGURE 1. (a) Histogram of all plasma vitamin A levels. (b) Histogram of the logarithms of the same values; the distribution is now normalized.

RESULTS

The distribution of plasma vitamin A levels amongst all specimens was markedly skew (Fig. 1a); this curve is, in fact, a very close approximation to a lognormal function (Fig. 1b). No significant difference was found between the plasma levels in males (mean value of all subjects 50·2 μg/100 ml) and those in females (mean value of all subjects 51·6 μg/100 ml).

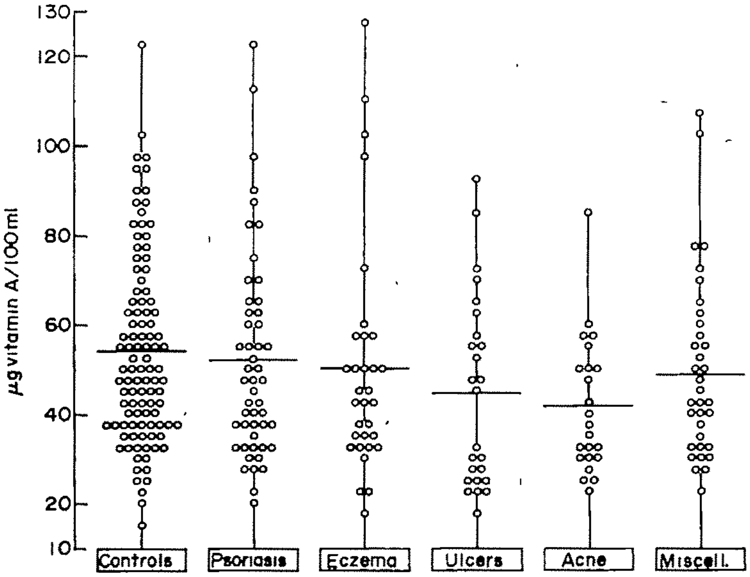

FIGURE 2. Distribution of plasma vitamin A levels according to clinical diagnosis; the mean values are indicated by horizontal bars.

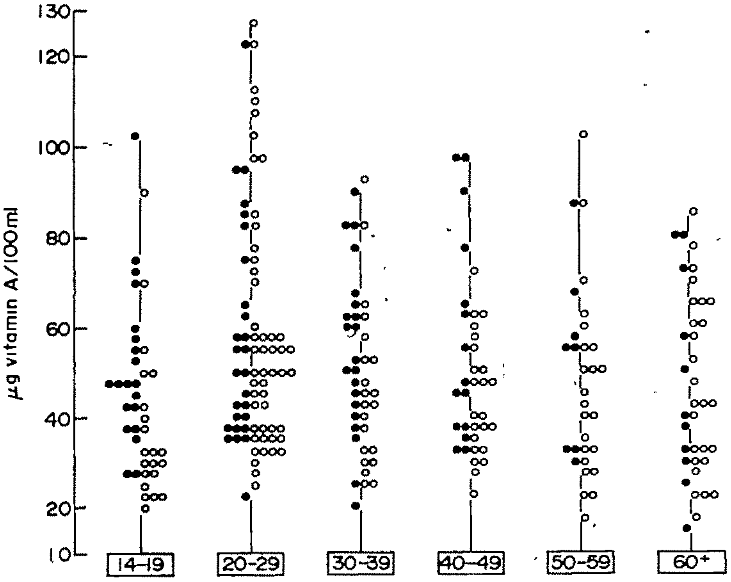

FIGURE 3. Distribution of plasma vitamin A levels according to age. The experimental subjects are represented by open circles and the control subjects by solid circles.

The mean plasma vitamin A level for the control group was 54·1 μg/100 ml, the range (95% confidence limits, calculated on the assumption of a lognormal distribution) being 23–110 μg/100 ml. The experimental group as a whole showed a slight trend towards lower values, with a mean of 48·7 μg/100 ml; plasma levels in the individual dermatoses are illustrated in Fig. 2.

The distribution of plasma vitamin A levels according to age is illustrated in Fig 3. Comparison of the experimental subjects with the control subjects within each age group (Wilcoxon rank test) showed no significant difference except in the youngest group (<20); here the lower vitamin A level in the skin patients was highly significant ($P<0·01$).

DISCUSSION

The colorimetric assays usually employed for vitamin A determination (mostly based on the reaction with antimony trichloride) are not entirely satisfactory. Their relatively low sensitivity, coupled with a somewhat doubtful specificity, tends to give particularly poor reproducibility over the lower part of the normal range. A fluorimetric approach overcomes these limitations; it is specific, exceedingly sensitive, and has a reproducibility of the order of 5% over the whole range.

Plasma vitamin A levels are, of course, closely related to dietary habits. We have therefore chosen to compare our 'experimental group' with patients attending another Department of the same hospital rather than with an arbitrary group of 'healthy subjects', in order to avoid the possibility of systematic bias in geographical or socio-economic parameters. The most closely comparable of recent investigations is perhaps that of Kahan (1969), who used an identical fluorimetric assay procedure to study a group of hospitalized patients in Sweden. Kahan also observed the distribution to be lognormal; he reported a mean value of 49 μg/100 ml (range 23–117 μg/100 ml) for subjects aged 18–45 years, figures which are remarkably similar to our present findings.

Reduced plasma vitamin A levels in patients with skin disease have been reported from time to time (Kühnau & Luniatschek, 1937; Schneider & Widder, 1938; Takonohashi, 1970). Other workers have denied this (Cornbleet, Popper & Steigmann, 1944; Leitner & Moore, 1946), and one investigation suggested that vitamin A levels may actually be raised in psoriasis (Hoffmann, Schneider & Quamo, 1950). Our own results clearly support the first of these groups. Classification of our figures according to disease (Fig. 2) yields little further information except that the downward trend is particularly marked in patients with acne or suffering from ulcers.

The atypical age distribution of these particular conditions (mean ages 20·9 years and 53·8 years respectively) suggested that the 'high-risk' group might be better defined in terms of age than in terms of diagnosis. This is indeed confirmed by Fig. 3, from which two points emerge clearly. In the older age-groups (50+) both the experimental and the control subjects show a slight decline in vitamin A levels; this is presumably a 'normal' phenomenon. In the youngest age-group (<20), on the other hand, there is a sharp and highly significant decrease in the vitamin A status of the experimental subjects only. The individual values within this latter group showed no correlation with clinical diagnosis. They were, however, related to the severity of the disease; in six out of seven of the young patients with a plasma vitamin A level of less than 30 μg/100 ml the lesions were both unusually extensive and of more than 2 years duration.

The interpretation of this finding must surely lie in what Shuster & Marks (1970) call 'the metabolic cost of skin disease'. It is clearly in the young patient, where there is already a high commitment of vitamin A for growth, that problems of 'metabolic payment' might be anticipated. The possible value of long-term vitamin A supplements for this high-risk group remains to be assessed.

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Angioleiomyoma of the skin

D.M.MACDONALD* AND K.V.SANDERSON

Skin Department, St George's Hospital, Hyde Park Corner, London SWIX 7EZ

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SUMMARY

The clinical and histological features of eighteen cases of angioleiomyoma are reported and the literature is reviewed. It is apparent that despite consistent characteristics these tumours continue to pose diagnostic problems to clinicians.

In the earlier part of the nineteenth century, angioleiomyoma, with glomus tumour and neuroma, was doubtless diagnosed as tuberculum dolorosum (Wood, 1812). The first definite report of a leiomyoma arising from a vessel wall was that of Aufrecht in 1868, although leiomyoma cutis had been described by Virchow in 1854. Besnier (1880) subdivided leiomyomata into leiomyoma cutis and dartoic myoma but ignored vascular leiomyoma. A more complete classification was published by Babes in 1884 and forms the basis of the varieties of leiomyoma recognized today. Only three of Ormsby's (1925) forty-four cases were solitary (and therefore likely to be vascular) which indicates that angioleiomyomata were not well recognized then. The characteristics of these tumours were brought to prominence by Stout (1937) who, at the end of a comprehensive review of solitary leiomyoma, suggested that a wider knowledge among clinicians of the existence of the tumours would lead to their more frequent recognition. The papers published since then have confirmed the distinctive features and relative frequency of angioleiomyoma, but have also shown that surgeons and dermatologists remain in ignorance of it, and that pathologists are not as adept at its diagnosis as they might be. Our main purpose in recording a series of eighteen cases seen at St George's Hospital in the past 10 years is to draw attention once again to a tumour which ought to be as easy to recognize as the glomus tumour.

METHODS

The case reports and histological sections of thirty-four cases with a pathological diagnosis of leiomyoma of the skin seen at St George's Hospital from 1963 to 1972 were examined. Of these, eighteen proved to be angioleiomyoma. The sections were of tumours excised surgically, fixed in neutralized 10% formol saline, embedded in paraffin and cut at 5 μm. Sections were stained with haematoxylin and eosin and, in some cases by van Gieson's method, by phosphotungstic acid–haematoxylin to show myofibrils and by the method of Gordon & Sweet for reticulin fibres.

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* Reprint requests to Dr D.M.MacDonald, Department of Dermatology, King's College Hospital, London, S.E.5.

RESULTS

The patients ages ranged from 24 to 80 years and only two were below the age of 30 years (Table 1). Sixteen (89%) of the cases were female. All the lesions were solitary; thirteen were situated around the knee or on the lower leg, three were situated around the elbow and one each on the lower lip and nose. Ten of the lesions were painful spontaneously or on pressure. The lesions on the face were not painful. The diagnosis was not made before either biopsy or excision in any of the cases, and in two the first biopsy failed because of the situation of the lesion deep in the subcutis.

TABLE 1. Clinical data.

Case no.	Year	Age (years)	Sex	Site	Clinical Diagnosis	Pain
1	1963	64	Female	(L) shin	Glomus tumour	–
2	1965	54	Female	(L) knee	? Epidermoid cyst	?
3	1966	75	Female	(R) shin	?	+
4	1966	59	Female	(R) knee	?	?
5	1969	58	Female	(R) shin	'Cyst'	+
6	1970	46	Male	Lower lip	Venous lake	–
7	1971	45	Female	(L) Lower leg	? 'Cyst' ? Fibroma	?
8	1971	31	Female	(R) shin	'Cyst'	?
9	1971	80	Male	(L) olecranon	Tophus	?
10	1971	56	Female	Sole of foot	Glomus tumour	+
11	1971	64	Female	(R) knee	'Cyst'	+
12	1971	30	Female	(L) elbow	? Ganglion ? Fibroma	+
13	1971	24	Female	(L) shin	? Glomus tumour ? Fibroma	+
14	1972	49	Female	(R) knee	? Fibroma	+
15	1972	55	Female	(R) elbow	Haemangioma	+
16	1972	59	Female	(R) ankle	Ganglion	+
17	1972	27	Female	(R) lower leg	? Ganglion ? Cyst	?
18	1972	60	Female	Nose	Neurofibroma	–

The microscopical appearance of the tumours was uniform in several respects. All were circumscribed and encapsulated, rounded in shape and situated at the level of the lower dermis or subcutaneous tissue (Fig. 1). Their main constituent was interlacing bands of smooth muscle cells which could be distinguished in H & E preparations by their brightly eosinophilic cytoplasm and baton shaped nuclei when cut lengthwise. All the lesions contained numerous vascular channels. Most of the vessels had a very prominent band of muscle in their walls. Although some had circular lumina, others had a slit-like or stellate lumen or showed evidence of occlusion or organization of a thrombus (Fig. 2). Areas of mucinous alteration occurred in the majority of tumours and were more prominent in the larger ones. In no tumour was a special stain necessary to distinguish the lesion from other dermal nodules. The combined features are unmistakeable. Three of the tumours had

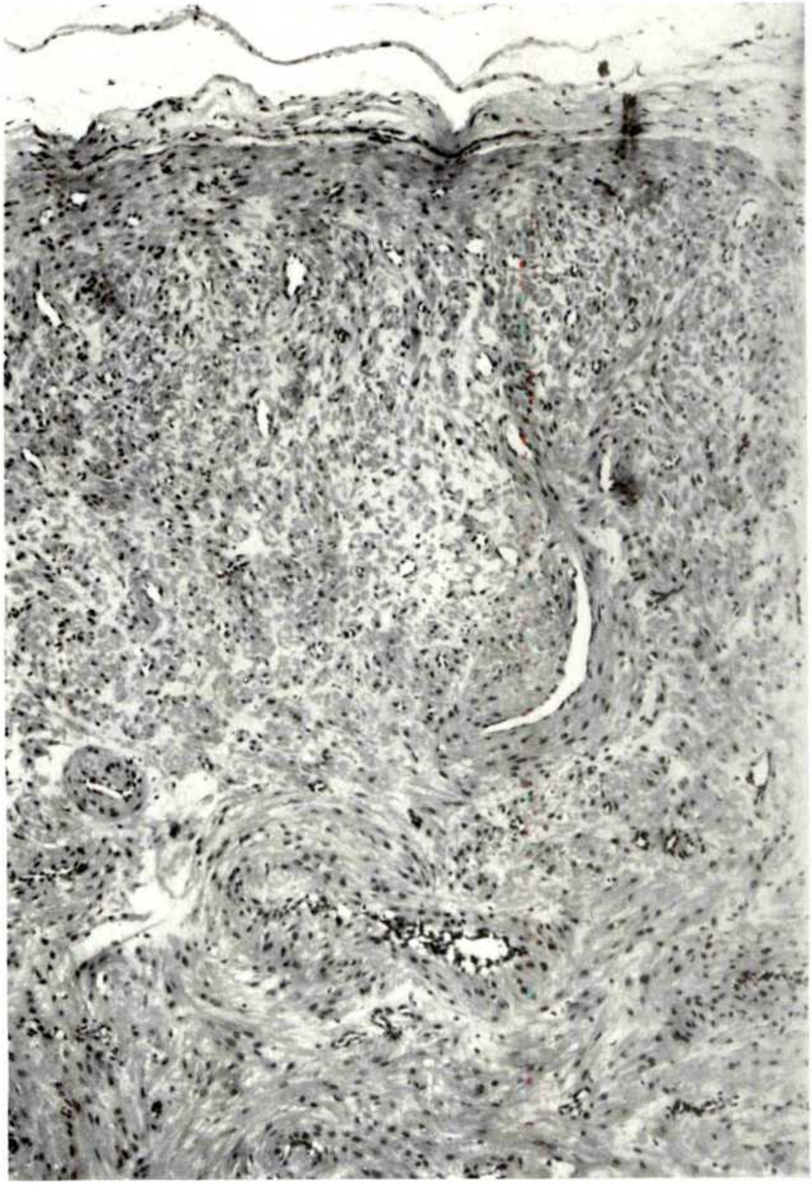

FIGURE 1. The tumour is rounded and encapsulated with dense bands of smooth muscle cells at the periphery and around many of the vessels. Elsewhere the cells are separated from each other by mucinous clefts. (H & E, × 50).

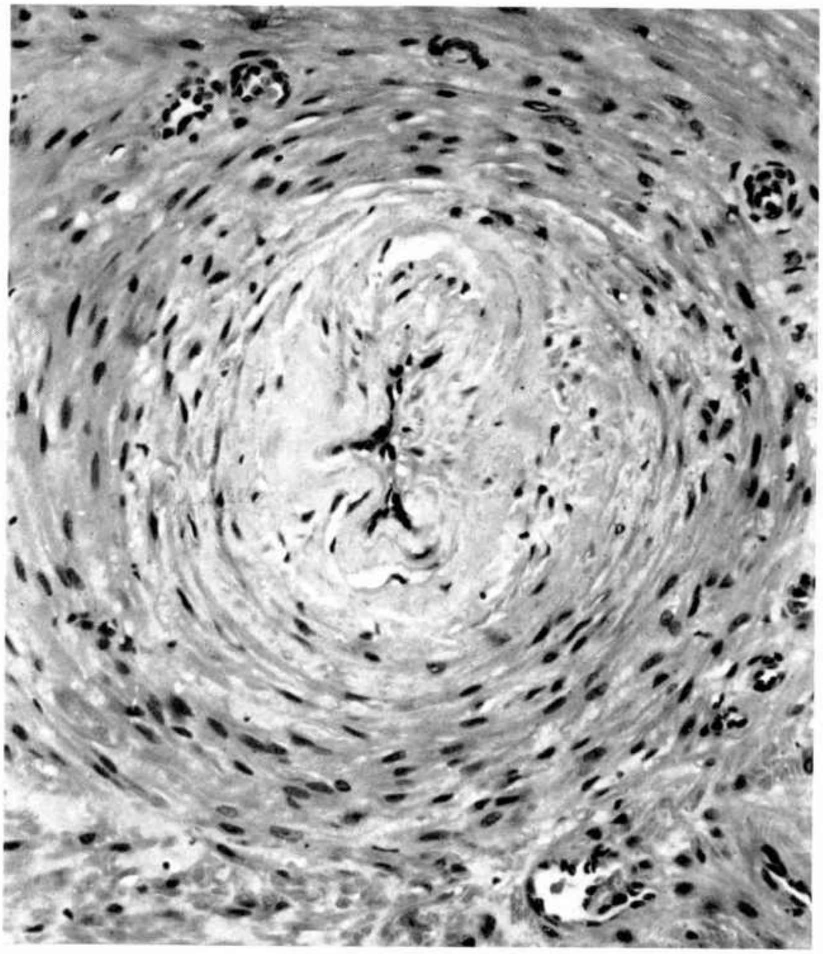

FIGURE 2. A vessel with encircling bands of smooth muscle and a compressed and rather stellate lumen (H & E, × 125).

epithelioid cells in the walls of some vessels resembling in some degree glomus cells (Fig. 3). There was no correlation between this appearance and a history of pain.

DISCUSSION

The clinical facts which emerge from this series agree with the more modern studies as illustrated by Table 2. The tumours occur predominantly in middle-aged females and are most frequently sited on the extremities, much more commonly on the legs than on the arms. The lesions are almost always solitary but one case with two lesions has been described (Duhig & Ayer, 1959). The entity of multiple vascular leiomyomata in several organs including the subcutaneous tissue recently described (Lin & Svobada, 1971) is totally different and is probably one of the juvenile fibromatoses (Stout, 1954). Pain, either spontaneous or provoked by pressure or cold, although considered to be characteristic, occurs in less than half the cases. Our experience and a review of previously recorded cases (Stout, 1937;

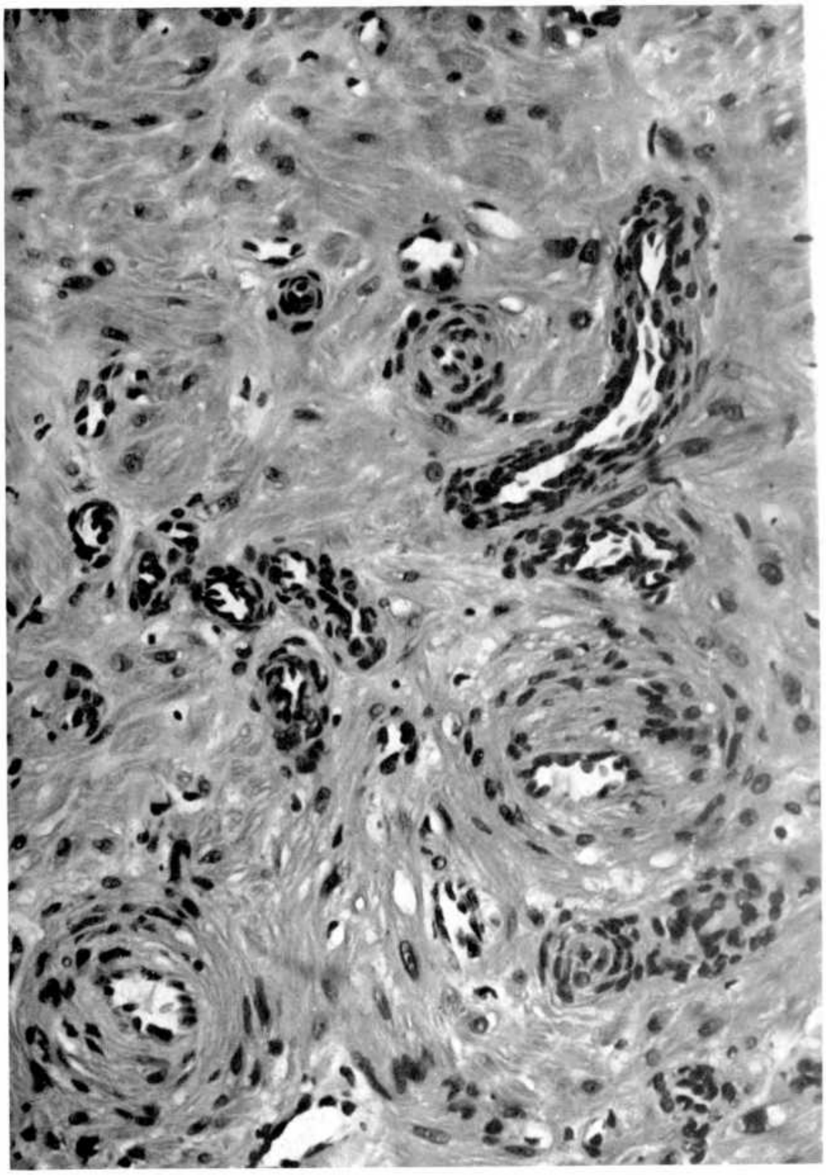

FIGURE 3. Vessels with smaller 'epithelioid' cells around them, resembling, in some degree, those seen in glomus tumour (H & E, × 125).

TABLE 2. Findings of modern studies.

Study	No. of cases	No. aged over 30	Female: male	Situation			No. painful
				Upper limb	Lower limb	Head	
Stout (1937)	12	?	7:5	3	9	—	6
Ekestrom (1950)	47	?	26:21	17	21	8	19
Auger (1951)	22	?	14:8	5	15	2	?
Jansen (1952)	4	2	2:2	1	3	—	3
Montgomery & Winkelmann (1959)	8	8	4:4	2	6	—	8
Duhig & Ayer (1959)	61	49	40:21	7	48	6	27
Magner & Hill (1961)	15	15	7:8	5	9	1	6
Present series	18	16	16:2	3	13	2	10

Ekestrom, 1950; Duhig & Ayer, 1959; Magner & Hill, 1961; McGowan & Harold Jones, 1969) suggest that angioleiomyomata on the face and possibly on the trunk are pain-free. The tumours are rarely larger than 4–5 cm diameter and, although vascular leiomyomata up to 30 cm diameter have been claimed (Drew, 1966), it is likely that these tumours were some other entity, e.g. neurilemmoma. Others have noted the almost invariable failure to make a correct clinical diagnosis (Macotela-Ruiz, 1963; Templeton, 1972), which must be due to lack of familiarity with the condition. Nevertheless, from the number of cases seen in this study in 1971 and 1972, we strongly suspect that the problem may be relatively common. Its incidence is almost certainly greater than that of glomus tumours (Duhig & Ayer, 1959), which are more readily diagnosed in the differentiation of small painful subcutaneous nodules. In fact, glomera are uncommon on the legs except subungually. The inference from the available information should be that a small painful subcutaneous nodule occurring on the lower limb of a middle-aged female will most likely be an angioleiomyoma. Ekestrom's (1950) comparison between glomus tumours and angioleiomyomata suggested that while they differed in that the latter are not found under the nails where glomera are numerous, nor have myelinated nerve fibres been shown with consistency in angioleiomyoma, they are similar in having epithelioid cells in their vessel walls and in lacking an elastic lamina. He postulated that the two lesions were probably closely associated and each was related to arteriovenous anastomoses. Indeed, one of Ekestrom's cases showed an angioleiomyoma and a glomus tumour in close juxtaposition. Other studies, which failed to demonstrate 'epithelioid' cells or neurofibrils (Magner & Hill, 1961; Saunders & Fitzpatrick, 1956), seem to deny this relationship. This question is further complicated by electron microscopic evidence which suggests that the 'epithelioid' cells of glomera are smooth muscle cells cut transversely (Murad, von Haam & Murthy, 1968; Venkatachalum & Greally, 1969; Tarnowski & Hashimoto, 1969).

The observation of Montgomery & Winkelmann (1959) of a pair of nerve trunks within the substance of an angioleiomyoma led them to believe that compression of somatic nerves accompanying the blood vessels within the tumour was the cause of pain. Other histological studies which failed to show nerve fibrils except in the tumour capsule (Stout, 1937; Duhig & Ayer, 1959; Ekestrom, 1950; Saunders & Fitzpatrick, 1956; Magner & Hill, 1961) would seem to refute this belief. The mechanism of pain production is obscure, although pressure on neurofibrils or contraction of vessels giving rise to local ischaemia may be involved. Most authors (Stout, 1937; Auger, 1951; Magner & Hill, 1961; Wilder & Lotfi, 1971) believe that angioleiomyomata arise from veins. Certainly the vessels within these tumours resemble veins more than arteries. Auger (1951) pointed out that neoplastic blood vessels may not faithfully reproduce either arteries or veins.

Duhig & Ayer (1959) suspected that pregnancy and sex hormone levels might play a part in the development of angioleiomyomata, because their age distribution is similar to that of uterine leiomyomata. Burrows & Horning (1952) have reported that fixation of oestrogen in traumatized tissue increases the rate of local mitosis. A past history of injury or leg eczema is commonly recorded and in at least four of this series the tumour developed at the site of previous damage. Templeton (1972) observed the remarkable frequency of this tumour in Uganda—fifteen times as common as glomus tumours, twenty-five times as common as leiomyoma cutis and one-third as common as Kaposi's sarcoma. Indeed, the prevalence in East Africa is ten times that of a Caucasian population living in temperate climates, which may be of epidemiological significance.

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Knotting of scalp hair

RODNEY P.R.DAWBER

Department of Dermatology, North Staffordshire Medical Centre,
Stoke-on-Trent

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SUMMARY

A study has been made of the incidence of knotting in scalp hair of various types. Short curly hair of negroid and caucasoid type appeared particularly disposed to knot. Scanning electron microscopy of affected hairs showed marked disruption of the hair shaft structure in and adjacent to the knots.

Reports of knotting of scalp hair (trichonodosis) have been very infrequent in the dermatology literature (Michelson, 1884; Galewsky, 1906; Pratt, 1947). It is generally considered to be rare and probably traumatic in origin. It rarely presents to dermatologists and no reference is made to this entity in several popular dermatology textbooks.

In this study evidence will be presented to show that knotting of hair is common and is frequently associated with disruption of the hair shaft structure.

PATIENTS AND METHOD

Eighty normal adult female subjects were studied, none having clinical evidence of hair or scalp disease or a history of excessive hair treatment with heat, bleaching or 'perming'. They were divided into four groups of twenty according to hair type: (1) negroes of American, West Indian or African origin with short, tightly-curled hair; (2) English subjects with (a) short curly hair, (b) long curly hair and (c) long straight hair. In each case the whole scalp was examined systematically with a ×8 magnifying lens for evidence of knotting. Hair shafts with knots were plucked and examined under the scanning electron microscope.

Results

Table 1 summarizes the results. The group of negro women showed the greatest number of knotted hairs whilst no knots were seen in English women with long straight hair. One of the latter group had two hair shafts in the frontal region each with one peripilar keratin cast. No hairs showed more than one knot.

Scanning electron microscopy

All the knotted hairs showed abnormalities localized to the part of the shaft in and around the knots (Fig. 1). The changes consisted of longitudinal fissuring and fracture of the cuticle; whole segments of

TABLE I.

Hair type	No. of subjects	No. showing knots	No. of knots per subject
Negroid	20	5	8 5 5 5 4
Caucasoid			
(a) Short and curly	20	3	5 4 4
(b) Long and curly	20	1	1
(c) Long and straight	20	0	(1 subject with 2 keratin casts)

cuticular scales were lost, exposing the underlying longitudinally-orientated cortical cells (Figs. 2 and 3). These changes are the same as those seen in trichorrhexis nodosa (Dawber & Comaish, 1970). No surface abnormalities were seen on the knotted shafts except adjacent to the knots, and hairs without knots were considered to be within normal limits.

Several normal hairs were knotted by the author and examined under the scanning electron microscope. Longitudinal fissuring of the cortex was seen in the knotted sections.

Of the forty-one knotted hairs examined, thirty-four were anagen hairs, three wave telogen hairs and four could not be classified.

Fig. 4 shows the keratin mass constituting one peripilar keratin cast.

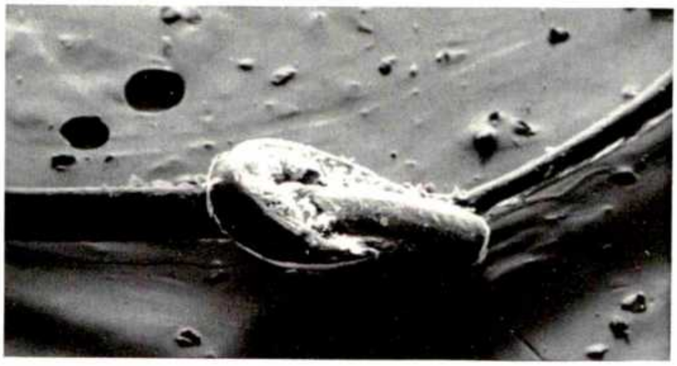

FIGURE 1. Knotting of hair. Scanning electron micrograph (× 110).

DISCUSSION

Fitzpatrick *et al.* (1971) stated that long curly hair knots most frequently, whilst Savill & Warren *et al.* (1962) suggested that short curly hair of relatively flat diameter is most often affected. The results of this study suggest that the latter is correct, although flattening of the shaft was not seen in the knotted hairs.

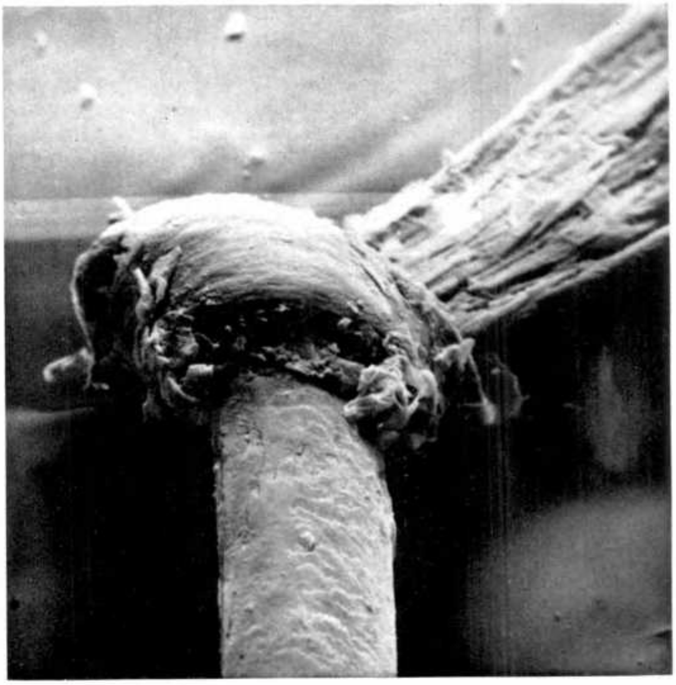

FIGURE 2. Knotting; showing cuticular scale loss and longitudinal fissuring below, and loss of cortical cells above the knot. Scanning electron micrograph (× 450).

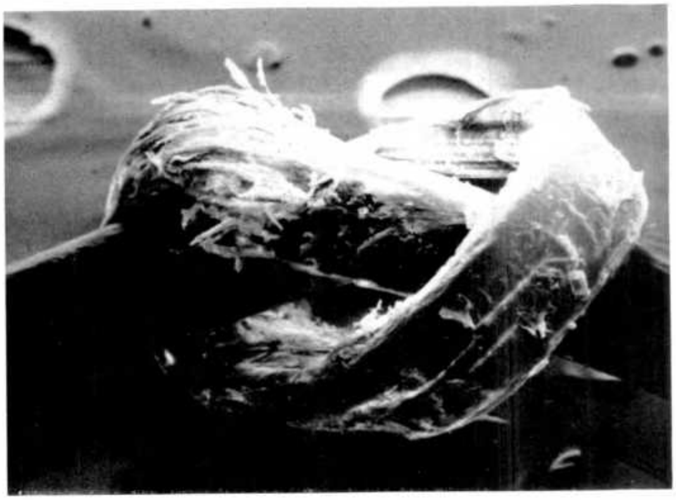

FIGURE 3. A single knot showing associated cuticular and cortical damage of trichorrhexis nodosa type (× 215).

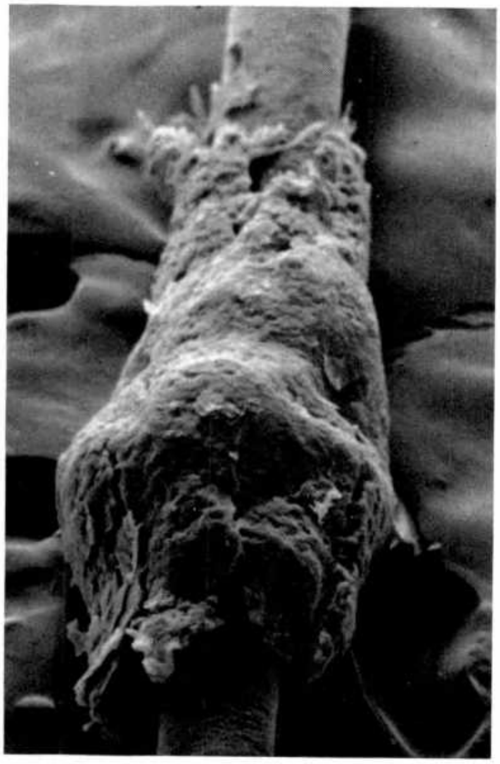

FIGURE 4. Peripilar keratin cast composed of a mass of keratin surrounding, but not attached to, the hair shaft, which appears normal (× 200).

Knotting almost certainly occurs with even greater frequency than shown here. Several hairdressers have suggested to the author that many are produced as 'slack' knots by friction on pillows and from various cosmetic procedures. Combing is thought both to tighten the knots and subsequently to pull out the affected hairs even in the anagen phase.

Disruption of the hair shaft is almost certainly secondary to the knotting, since (a) hairs without knots showed no signs of abnormal cuticular or cortical damage, (b) the knotted shafts were damaged only close to the knots, unlike trichorrhexis nodosa in which the whole shaft is abnormal apart from the proximal 0·5–1 cm, and (c) the hair cuticle of normal hairs on which knots were tied showed cuticular fissuring similar to the abnormal hairs.

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Antibodies to native DNA in discoid lupus erythematosus

P.DAVIS,* BRENDA ATKINS AND G.R.V.HUGHES

Department of Rheumatology,
Royal Postgraduate Medical School,
Ducane Road, London, W.12

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SUMMARY

DNA antibodies, known to be highly specific for systemic lupus erythematosus, have been measured in twenty-seven cases of discoid lupus erythematosus, without evidence of systemic involvement. In seven (27%), DNA antibodies have been found in the range normally associated with active SLE. It is suggested that these results support the concept that discoid LE and SLE are closely related diseases, and that the finding of DNA antibodies in patients with discoid LE may be of value in management and prognosis.

The exact relationship between discoid lupus erythematosus and systemic lupus erythematosus (SLE) is still in doubt. The high incidence of discoid lesions in patients with SLE, the progression of between 1·3% and 5% of cases of discoid LE to SLE (Cannon & Curtis, 1958; Scott & Rees, 1959) and the identical histological nature of the discoid lesions in both diseases has led to the belief that discoid LE and SLE are variants of the same disease with a similar pathogenesis (Gold, 1967; Dubois, 1966). Burch & Rowell (1968, 1971), however, have provided evidence to suggest that discoid LE and SLE are separate disease entities with different genetic background and natural history. Serologically, the main difference between SLE and discoid LE appears to be only in the incidence in which abnormalities are detected; LE cells, antinuclear antibodies, rheumatoid factor, false positive WR, and positive Coombs tests occur in both diseases but much less frequently in discoid LE than SLE (Rowell, 1971; Beck & Rowell, 1966).

The use of the Farr technique for the measurement of anti-DNA antibodies has provided a therapeutic guide to disease activity in SLE (Hughes, Cohen & Christian, 1971). In earlier studies (Hughes, 1971), it was noted that DNA antibody titres were normal in a small number of patients with discoid LE. In one discoid LE patient, a rise in titre of DNA antibodies paralleled the development of systemic features. This observation prompted us to study anti-DNA antibodies in discoid LE and to correlate the results with the extent of systemic involvement.

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* Reprint requests should be addressed to Dr P. Davis, Department of Medicine, Bristol Royal Infirmary, Bristol.

TABLE I. Clinical features of forty-two cases of discoid LE

Patient	Sex	Age (years)	Duration (years)	LE	ANF	Other features	Biopsy	ARA Crit.	R_x	Max. DNA
Group 1										
P.F.	F	37	5	+	+	Arthritis, alopecia, chorea	+	+	Prednisone	70%
D.P.	F	25	4/12	Neg.	+	Arthritis, alopecia		+	Prednisone	66%
D.H.	F	21	10/12	+	+	Alopecia, photosensitivity, nephritis, mouth ulcers, thrombocytopenia		+	Prednisone	95%
J.W.	F	26	2	Neg.	+	Arthritis, alopecia, photosensitivity	+	+	Prednisone	83%
I.M.	F	55	16	+	+	Arthritis, Raynaud's, alopecia	+	+	Prednisone	52%
E.W.	F	43	18	+	+	Arthritis, Raynaud's, butterfly rash	+	+	Betnovate	70%
N.D.	M	45	4	Neg.	+	Alopecia, arthritis, pleurisy, pericarditis		+	Prednisone	80%
H.S.	F	38	7	Neg.	+	Raynaud's, alopecia, arthritis leukopenia		+	Prednisone	75%
V.S.	F	51	20	+	+	Arthritis, nephritic syndrome, pleurisy		+	Prednisone	41%
R.B.	F	35	3	Neg.	+	Alopecia, photosensitivity, arthritis, leukopenia	+	+	Prednisone, Azathioprine	63%
M.C.	F	24	7	+	+	Arthritis, convulsions		+	Prednisone	90%
M.L.	F	46	7	+	+	Alopecia, arthritis, nephritic syndrome, haemolytic anaemia	+	+	Prednisone, azathioprine	39%
S.B.	F	38	3	Neg.	+	Alopecia, arthritis, nephritic syndrome, pleurisy, psychosis		+	Prednisone, azathioprine	73%
F.B.	F	46	4	+	+	Arthritis, nephritic syndrome, pleurisy, haemolytic anaemia		+	Prednisone	75%
T.L.	F	16	1	+	+	Raynaud's, alopecia, pleurisy, photosensitivity, leukopenia		+	Prednisone	40%

Group 2										
E.M.	F	47	20	–	+	Leukopenia		–	Synalar forte	35%
A.W.	M	48	4	–	+		+	–	Mepacrine	34% (35%)
A.M.	M	63	2	–	–		+	–	Propaderm	7%
J.G.	F	20	4	–	–		+	–	Betnovate	27%
V.D.	F	43	11	–	–		+	–	Synalar forte	14%
R.D.	M	49	4	–	–		+	–	Betnovate	9%
T.R.	F	42	4	–	–		+	–	Synalar	9%
E.B.	F	66	20	+	+			–	Betnovate	76%
J.P.	F	50	8	–	+		+	–	Chloroquine	45%
F.H.	F	39	18	–	+		+	–	Betnovate, prednisone	50%
D.N.	F	49	18	–	–	Rheumatoid arthritis		–		0%
S.B.	F	36	6	–	–		+	–	Betnovate	26%
B.B.	F	35	6/12	–	–			–	Chloroquine	26%
F.C.	F	76	1	–	–			–	Betnovate	12%
O.H.	F	49	21	–	+		+	–	Metosyn	38%
M.S.	F	53	20	–	+			–	Metosyn (steroids/ azathioprine)	29%
E.H.	F	51	4	–	–			–	Metosyn	23%
N.H.	F	54	19	–	–			–	Hydrocortisone	8%
J.B.	F	43	8	–	–			–	Betnovate	41%
A.M.	F	47	9	–	+		+	–	Uvistat	11%
E.W.	F	65	1	–	–			–	Chloroquine/ Betnovate	12%
D.R.	F	33	10	–	–			–	Chloroquine/ Metosyn	21%
M.C.	F	52	11	–	–		+	–	Triamcinolone	9%
B.A.	F	42	7	–	–			–	Chloroquine	24%
B.S.	F	37	3	–	–		+	–	Betnovate	14%
J.B.	F	43	2	–	–		+	–	Chloroquine	40%
P.C.	F	35	2	–	–		+	–	Synalar	23%

CASES

Forty-two patients with discoid LE have been examined in this study. These have been divided into two groups: fifteen patients with systemic manifestations (Group 1) and twenty-seven with discoid lesions alone (Group 2).

The clinical and laboratory features of all cases are presented in Table 1. All cases with systemic involvement satisfy the American Rheumatism Association's preliminary criteria for the classification of SLE (Cohen *et al.*, 1971b; Davis *et al.*, 1973). Twenty normal sera were used as controls.

Of the group with systemic involvement, three are known to have presented with discoid LE prior to systemic features. These three cases are reported later.

METHOD

Farr technique (Cohen *et al.*, 1971a)

0·05 ml of serum is diluted 1:10 in borate buffer (pH 8·0). 0·05 ml of diluted serum is then mixed with 0·05 ml of ^{14}C DNA (Radiochemical Centre, Amersham) and incubated for 1 h at 37°C. Samples are then left overnight at 4°C. The samples are kept on ice and 0·1 ml of saturated ammonium sulphate added and left at 4°C after thorough mixing for 1 h. Samples are then spun for 45 min at 2000 rev/min at 4°C. 0·1 ml of supernatant (S) is removed and added to 0·9 ml of borate buffer followed by 10 ml of Bray's scintillation fluid. Similarly, the remaining 0·1 ml containing the precipitate (p) is diluted with 0·9 ml of borate buffer and 10 ml of Bray's fluid added. Radioactivity in supernatant (S) and precipitate (p) is measured in counts per minute (ct/min) on a β-emitter counter.

DNA binding is calculated by: (ct/min (p) − ct/min (S))/(ct/min (p) + ct/min (S)) and expressed as a percentage. Normal range in our laboratory is 0–30%.

RESULTS

Maximum DNA binding results recorded in each patient have been plotted (Fig. 1). All cases in whom systemic features were present had DNA binding values greater than 30% (range 39–93%, mean value 67%). All the normal controls had values below 30% (range 0–27%, mean 16%).

Seven of the twenty-seven patients with only skin lesions (27%) had DNA binding values greater than 30%, falling within the range usually associated with active SLE. The remaining cases had values within the normal range. There were no clinical features in patients with discoid LE to distinguish those with high DNA binding from those in whom it was normal.

CASE REPORTS

Case 1

Miss P.F. developed discoid LE in January 1968 at the age of 32. Diagnosis was confirmed on biopsy. She was successfully treated with topical steroids and later chloroquine. In March 1971 she developed a recurrence of her rash associated with arthritis, alopecia, visual disturbance and chorea.

Investigations showed: ESR 65 mm/h, LE cells positive, ANF strongly positive, DNA antibodies positive (63%). The patient responded to oral steroids with regression of symptoms and a fall in DNA antibodies to within normal limits.

Case 2

Mrs I.M. developed discoid lupus erythematosus, confirmed by biopsy, at the age of 39. She was

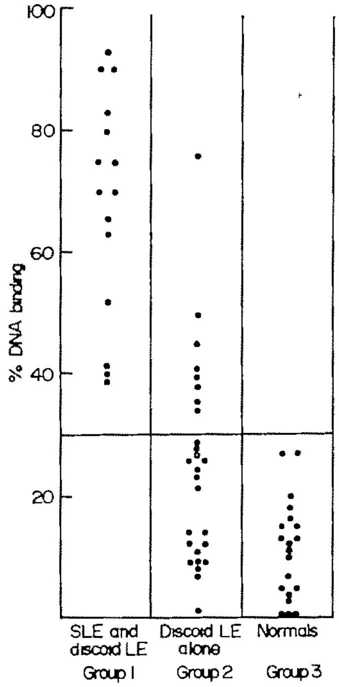

FIGURE 1. Maximum DNA binding results.

treated with topical steroids and the disease ran an uncomplicated course for 15 years. In May 1972 she developed a non-deforming arthritis, Raynaud's phenomenon, and diffuse alopecia.

Investigations showed: ESR 72 mm/h, leukopenia with absolute lymphopenia, LE cells positive, ANF positive 1:160. DNA binding 52%. The patient improved on oral steroids, with a fall in DNA binding to normal.

Case 3

Mrs E.W. developed discoid LE with typical histological changes in 1956 at the age of 23. Her skin lesions ran a chronic course with repeated relapses following remissions induced with topical steroids, hydroxy chloroquine and CO_2 snow. In April 1972, 16 years later, during a severe exacerbation of her discoid lesions, a butterfly rash, non-deforming arthritis and Raynaud's phenomenon were noted.

Investigations showed: ESR of 43 mm/h, positive LE cells and ANF. DNA binding level was 70%.

DISCUSSION

DNA antibodies have hitherto been regarded as being highly specific and sensitive for SLE. In particular, they are rarely found in other conditions, such as Sjøgren's syndrome, chronic active hepatitis and Felty's syndrome, commonly associated with positive ANF and LE cells (Hughes, 1973). In our results the mean DNA binding value of patients with discoid LE was higher than that obtained in these other conditions. A similar raised mean DNA binding in patients with discoid LE has been reported by Mandel *et al.* (1972). Antibodies to DNA in patients with discoid LE have also been noted by Sylvester *et al.* (1973) in their study of antibodies to RNA which were present in 42% of cases. Preliminary studies in our department have confirmed this finding in 20% of the cases examined.

Of interest was the finding of a number of discoid LE sera with high DNA binding values in the range normally found in active SLE. In none of these patients was there clinical evidence of systemic involvement. The significance of high DNA binding values in SLE patients in clinical remission has been studied by Lightfoot (1972), who found them to predict exacerbation of disease activity in fourteen of thirty-two cases studied. While none of our patients has yet developed evidence of systemic involvement, it remains possible that they may subsequently develop multisystem disease as in the three cases reported above.

A further similarity between patients with discoid LE and SLE is the tendency of DNA antibody levels to fall with decreasing disease activity. In patient A.W., for instance, DNA binding fell from 34% at a time when florid skin lesions were present to 8% when lesions had disappeared after 8 weeks treatment with mepacrine. Further elucidation of these results will be required before their full significance can be determined. They do, however, suggest that DNA measurements may be a useful test in the management and prognosis of discoid LE, the ANF being a non-specific but valuable screening test.

In summary, our results lend support to the concept that discoid LE and SLE are a closely related disease entity. The suggestion, however, that these results support the possible role of a latent virus in the pathogenesis of these diseases (Talal, Steinberg & Daky, 1971) remains as speculation.

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Allergic contact dermatitis from the radish, *Raphanus sativus*

J.C.MITCHELL AND W.P.JORDAN

Division of Dermatology, The University of British Columbia, Vancouver 8, Canada, and the Department of Dermatology, Medical College of Virginia, Richmond, Virginia 23219, U.S.A.

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SUMMARY

A waitress, aged 38 years, developed acute contact dermatitis of her hands after chopping salad plants. Patch tests to radish (*Raphanus*) and to allyl *iso*thiocyanate and benzyl *iso*thiocyanate produced positive reactions. Sinigrin, the thioglucoside of allyl *iso*thiocyanate, was mixed in petrolatum with the enzyme myrosinase; the mixture produced a positive patch test reaction. Based on these preliminary findings, the immunochemical requisites of *iso*thiocyanates (mustard oils) are discussed. A tabulation has been made of exposure risk to plants of the mustard family (Cruciferae) which yield these compounds; the plants include some commonly used culinary vegetables, salad plants, condiments and also horticultural plants.

The radish, *Raphanus sativus* L., is a member of the Mustard family of plants (Cruciferae) (Willis, 1966), and the root material and seeds yield mustard oils (*iso*thiocyanates) (Kjaer 1963, 1966; Underhill & Wetten, 1966). The irritant effects of mustard are well known, both as a condiment and by the effects of mustard-plasters applied as rubefacients. Mustard oil has been reported to produce allergic contact dermatitis in the guinea-pig (Landsteiner & Di Somma, 1938) and in man (Gaul, 1964). A case is reported of contact sensitivity to the radish, with identification of relevant sensitizing chemicals; the risk of exposure to some plants which yield such mustard oils is presented in tabular form. In addition, what is perhaps the first demonstration of a substrate–enzyme patch test reaction has been carried out.

MATERIALS AND METHODS

Closed patch tests were applied to the skin of the upper back, and scored at 48 h by the method of Wilkinson *et al.* (1970). Allyl *iso*thiocyanate was purchased from Matheson, Coleman and Bell, Norwood, Ohio, U.S.A. Benzyl *iso*thiocyanate, phenyl *iso*thiocyanate, sinigrin and myrosinase were donated by E.W. Underhill, Ph.D., Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada.

One percent in alcohol was recommended as a suitable concentration and vehicle for patch testing with synthetic oil of mustard (Rostenberg & Sulzberger, 1939). Dr Howard Maibach (1972, written communication) carried out tests for irritancy, using petrolatum as a vehicle, with the following results: allyl *iso*thiocyanate 0·05% and benzyl *iso*thiocyanate 0·1% in petrolatum were non-irritant in four male controls. Phenyl *iso*thiocyanate 0·1% in petrolatum gave equivocal responses in two of the four individuals, and was considered to be a marginal irritant. Accordingly, phenyl *iso*thiocyanate was diluted to 0·05% in petrolatum for patch test purposes.

CASE REPORT

A 38-year-old black female waitress developed an acute vesiculo-bullous dermatitis of both palms; the sides of the fingers were more severely involved. Three weeks before the onset of the dermatitis she was assigned the task of chopping tomato, cabbage, endive and radish for a new salad-bar at the restaurant in which she worked. Two weeks after the dermatitis appeared she sought medical attention. The physical findings were normal with the exception of the hands. She denied past dermatological problems or significant medical illness.

Patch test results are recorded in Table 1. Positive reactions were observed to the root of a radish of the variety which the patient handled at work; by 'root' is indicated the edible bulbous underground portion. Patch tests to cabbage leaf (*Brassica*), tomato (*Lycopersicon*) and endive (*Cichorium*) were negative. Positive patch test reactions were observed to allyl *iso*thiocyanate and to benzyl *iso*thiocyanate: tests were negative to phenyl *iso*thiocyanate. Samples of the thioglucoside, sinigrin, which yields allyl *iso*thiocyanate, and of the enzyme, myrosinase, which is responsible for the hydrolysis of sinigrin, were mixed together and one week later the mixture was applied to the skin; a positive reaction was obtained. There was no reaction to freshly mixed sinigrin and myrosinase.

DISCUSSION

Numerous authors have described the irritant effect on the human skin of plants which yield mustard oils, but, apart from the conclusive report of Gaul (1964), no reports have been found which are detailed enough to confirm allergenicity, though some suggest such an effect.

A vesicular eruption of the hands, which occurred twice in a baker, was attributed to ground mustard, which was one of the materials he had handled (Tara, 1939); patch tests were not recorded. Shelmire (1940) observed in housewives contact dermatitis of the hands from mustard greens (*Brassica*), radish (*Raphanus*) and water-cress (*Nasturtium officinale*), but he gave no clinical details. Tamara oil (derived from *Eruca*, Cruciferae) has been reported to cause allergic and photodermatitis affecting the scalp, neck and ear region (Behl *et al.*, 1966). Some housewives handling radish leaves are sensitive and prone to get dermatitis of the hands (Behl *et al.*, 1966). Children playing with radish leaves in kitchen-gardens have been observed to develop dermatitis on the dorsa of the hands and on the forearms (Behl, unpublished, cited by Behl *et al.*, 1966).

Oppenheim's meadow dermatitis is evoked by psoralen-containing plants. It is possible that some early reports implicating cruciferous plants as agents of this clinical syndrome were in fact instances of an irritant effect enhanced by sunburn.

CHEMICAL AND BOTANICAL ASPECTS

The *iso*thiocyanate-producing glucosides (thioglucosides) constitute a well defined class of natural products occurring in a large variety of higher plants, which belong to a relatively small number of botanical families (Kjaer, 1960; Ettlinger & Kjaer, 1963). They are characterized by their ability

TABLE 1. Patch test results in a woman, aged 38 years, with allergic contact dermatitis from radish, *Raphanus sativus*

Patch test material/compound		Concentration % /vehicle	Results scored by method of Wilkinson *et al.* (1970)
Botanical name	Common name		
Raphanus sativus	Radish, root	as is	++ to +++ (spreading reaction)
Lycopersicon lycopersicum	Tomato, fruit	as is	–
Cichorium endivia	Endive, leaf	as is	–
Brassica oleracea var. *capitata*	Cabbage, leaf	as is	–
Chemical name	Chemical formula		
Allyl *iso*thiocyanate	$CH_2{=}CHCH_2N{=}C{=}S$	0·1, petrolatum	++ to +++ (spreading reaction)
Benzyl *iso*thiocyanate	$C_6H_5CH_2N{=}C{=}S$	0 1, petrolatum	++
Phenyl *iso*thiocyanate	$C_6H_5N{=}C{=}S$	0·05, petrolatum	±
Sinigrin (yields allyl *iso*thiocyanate)	Glucoside of $CH_2{=}CHCH_2N{=}C{=}S$	1·0, petrolatum	–
Myrosinase	enzyme	1·0, petrolatum	–
Sinigrin, 1·0 per cent in petrolatum mixed with myrosinase 1·0 per cent in petrolatum 1 week prior to application to the skin			++
Sinigrin 1·0 per cent in petrolatum mixed with myrosinase 1·0 per cent in petrolatum immediately prior to application to the skin			–

to undergo enzymatic hydrolysis to *iso*thiocyanates (mustard oils), hydrogen, sulphate and D-glucose (Kjaer, 1966), for example:

$$\begin{array}{c}\text{Sinigrin}\\ \text{(isothiocyanate glucoside)}\\ \text{(a thioglucoside)}\end{array} \xrightarrow[\text{(enzyme)}]{\text{myrosinase}} \begin{array}{c}\text{allyl \textit{iso}thiocyanate}\\ CH_2 = CHCH_2NCS\end{array}$$

Sinigrin has been found (in trace amounts) in the seeds of *Raphanus sativus* var. *radicula* and in the plants tabulated in Table 2 (Kjaer, 1960). *Raphanus sativus* varieties yield from their roots and seeds other mustard oils, namely *iso*propyl *iso*thiocyanate, $CH_3CH(CH_3)NCS$ and 4-methylsulphinylbutyl *iso*thiocyanate, $CH_3SO(CH_2)_4NCS$ (Kjaer, 1960). Gluocotropaeolin, which yields benzyl *iso*thiocyanate, $C_6H_5CH_2NCS$, has been found in some other plants, listed in Table 2 (Kjaer, 1960).

Nearly fifty thioglucosides have been isolated from about 350 species of plants in nine families (Kjaer, 1963). The majority belong to the Mustard family (Cruciferae); of more than 200 species of this family so far studied, hardly any have been devoid of thioglucosides; it seems safe, therefore, to conclude that glucosides of this type may be constant constituents of species of this family. The large Caper family (Capparidaceae) (Willis, 1966), which is a tropical relative of the Mustard family, is an equally consistent source of thioglucosides. The occurrence of more than one thioglucoside in a given plant species is the rule rather than the exception; as many as eight individual thioglucosides have been clearly distinguished in a single seed specimen (Ettlinger & Kjaer, 1963). Within the thioglucoside-containing species, the compounds seem often to be distributed throughout the plant. The total and relative amounts of the individual thioglucosides in various parts of the plant vary during its growth cycle. The amounts of thioglucoside in a plant vary from a trace to several per cent of the dry weight; in the case of fresh horse-radish (*Armoracia*), 3 kg of fresh roots, containing 60–70% water, afforded 10·6 g of analytically pure sinigrin (Ettlinger & Kjaer, 1963).

Phenyl-mustard oils were shown to be more irritant for the skin and conjunctivae of dogs than were aliphatic mustard oils (Takahashi, 1939). Our experience seems to indicate that this may be true also for the human skin.

Among the naturally occurring alkyl *iso*thiocyanates, both saturated and unsaturated compounds, e.g. allyl *iso*thiocyanate, are known to occur. Our findings seem to suggest that unsaturation β, γ- to the *iso*thiocyanate nitrogen (as in allyl *iso*thiocyanate and benzyl *iso*thiocyanate) is an immunochemical requisite, whereas α, β- unsaturation is not so; the latter compound (phenyl *iso*thiocyanate) has greater capacity for irritation (Fig. 1).

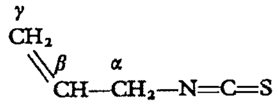
The functional binding group to the presumed carrier protein may be $-N = C = S$, and haptenic activity may reside at a specific distance, perhaps two carbon atoms away. Investigation of additional compounds is required to resolve this point. It will be of interest to determine whether $CH_3-N = C = S$ derived from plants of the Capparidaceae family (Mitchell, 1974) is allergenic. If β, γ unsaturation is indeed a requisite, this compound, while certainly irritant, may not be allergenic.

It would be desirable also to test with 4-methylsulphinylbutyl *iso*thiocyanate, which is derived from the roots and seeds of *Raphanus sativus* varieties (Kjaer, 1960). The positive patch test which we obtained to allyl *iso*thiocyanate does not necessarily indicate the presence of this compound in radish root but may indicate a cross sensitivity between allyl *iso*thiocyanate ($CH_2 = CHCH_2NCS$) and 4-methylsulphinylbutyl *iso*thiocyanate ($CH_3SO(CH_2)_4NCS$).

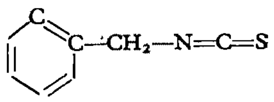
The known irritant effects of mustard oils may have over-shadowed their possible allergenicity and, certainly, caution must be exercised in selecting a non-irritant dose for patch testing. Quantitative patch testing is also desirable. The observation that patch testing with a substrate (sinigrin) together with an enzyme (myrosinase) produced a positive patch test reaction, whereas separate tests with the substrate and the enzyme produced negative reactions, lends itself to further quantitative investiga-

TABLE 2. Botanical sources of allyl *iso*thiocyanate and benzyl *iso*thiocyanate (Kjaer, 1960)

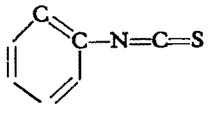
Compound	Botanical source		Exposure risk
	Botanical name	Common name	
Allyl *iso*thiocyanate	*Alliaria officinalis*	Wild Garlic	Wild plant
	Alyssum spp.	Madwort	Wild plant
	Armoracia lapathifolia	Horseradish	Condiment
	Barbarea vulgaris	Winter Cress	Salad plant
	Brassica juncea	Mustard greens	Vegetable
	Brassica nigra	Black mustard	Condiment
	Brassica oleracea var. *capitata*	(root) Cabbage	Vegetable
	Brassica oleraceae var. *hort*	Kohlrabi, Kale, Brussels sprouts, Cauliflower, Broccoli	Vegetables
	Cakile maritima	Sea Rocket	Sea-shore plant
	Capsella bursa-pastoris	Shepherd's Purse	Weed
	Crambe maritima	Sea Kale	Vegetable
	Diplotaxis muralis	White Wall Rocket	Weed
	Erucastrum gallicum		Weed
	Erysimum cheiranthoides & spp.	Blister Cress	Salad plant
	Hesperis matronalis	Rocket	Garden plant
	Iberis sempervirens	Candytuft	Garden plant
	Sinapis arvensis	Charlock, Wild Mustard	Weed
	Sisymbrium spp.	Hedge Mustard	Weed
	Thlaspi arvense	Penny Cress	Wild plant
Benzyl *iso*thiocyanate	*Alliaria officinalis*	Wild Garlic	Wild flower
	Cardamine graeca	Bitter Cress	Some species of the genus cultivated
	Coronopus didymus	Wart Cress	Some species of the genus cultivated
	Lepidium sativum	Garden Cress	Salad plant
	Lepidium spp.	Cow Cress, Pepper Grass	Weed
	Reseda lutea (Resedaceae)	Mignonette	Pot-plant, etc.
	Tropaeolum majus (Tropaeolaceae)	Nasturtium of gardens	Garden plant



Allyl *iso*thiocyanate



Benzyl *iso*thiocyanate



Phenyl *iso*thiocyanate

FIGURE 1. Molecular structure of the *iso*thiocyanates which were used for patch testing.

tions for irritancy and allergenicity. In view of the risk for gardeners and especially for housewives of exposure to plants which yield *iso*thiocyanates, as is evident from Table 2, further investigation for allergenicity of this group of compounds could be informative. The Mustard family of plants consists of 3200 species in 375 genera and is of cosmopolitan distribution, chiefly in the north temperate zone (Willis, 1966). An historical point may be relevant; the dye, woad, which was used by the Ancient Britons to colour their bodies, is derived from *Isatis tinctoria* (Cruciferae). The plant, which is still occasionally grown in gardens, yields mustard oils (Kjaer, 1960) and for dyeing purposes, the leaves were ground up into a paste and then fermented (Willis, 1966). It would be interesting to find out if sauerkraut, which is partially fermented and pickled cabbage (*Brassica*) has less effect on the skin than fresh cabbage. Cabbage root yields an allergenic *iso*thiocyanate; our negative patch test was obtained with a leaf of this plant.

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REM syndrome: reticular erythematous mucinosis (round-cell erythematosis), a new entity?

GERD KLAUS STEIGLEDER, HEINZ GARTMANN AND UTE LINKER

Department of Dermatology (Head of Department: Professor Dr G.K. Steigleder),
University of Cologne, Josef-Stelzmann-Strasse 9, D 5000 Köln 41, Germany

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SUMMARY

Four patients, three females and one male, were observed in Cologne with sheet- or net-like erythema of the upper chest and of the back, with a tendency to spread to the abdomen. The patients had no symptoms with the exception of itching in one case and itching after sunburn in another. Histologically the epidermis was normal, apart from slight hydropic degeneration and some minor degree of spongiosis. The main features were: dilated blood vessels in the dermis, with a more or less pronounced perivascular infiltrate of round cells, and, in addition, deposits of a substance which stained with alcian blue. Oral treatment with Rhetis was of some benefit, otherwise therapy, including corticosteroids, was not successful. Other antimalarial drugs were not used. There was some relation to sun exposure, but the full spectrum of xenon-light did not cause any abnormal reactions. Since it was not possible to relate these findings to any known syndrome, a combination of the main signs, reticular erythema and mucinosis, was used to name this entity REM syndrome.

Since 1970 we have treated four patients, three women and one man, suffering from a dermatosis hitherto unknown to us. We could find no description of any similar condition in the literature. On 20 October 1971, three of the patients were presented by Lischka & Orthenberger (1972) at the 19th Meeting of Cologne Dermatologists (Kölner Dermatologenabend). As a preliminary diagnosis, Steigleder coined the term round-cell-erythematosis (REM syndrome), as he felt that the patients represented a new entity. We found in our files records of a fifth patient with the same syndrome, a 48-year-old man who was treated in 1968 for another condition and also had REM syndrome. As he did not undergo the same tests as the other four patients and was not treated for REM syndrome, he has not been included in the present study, but he is referred to in another paper (Steigleder, Gartmann & Linker, 1974).

The dermatosis was located on the chest and on the upper part of the back, though the neck and abdomen were sometimes affected. In all four patients the disease started on the chest, with more or less sharply outlined sheet- or net-like erythema. The centre was slightly infiltrated. In differential diagnosis we considered a drug eruption, light eruption, and deposits caused by a metabolic disorder. Histologically, in all cases we found more or less pronounced perivascular infiltrates of round cells, most probably lymphocytes, and, in addition, deposits of a substance which stained with alcian blue in the dermis. The mucous membranes, genitalia, internal organs, and lymph nodes were found to be unaffected. None of the patients complained of any symptoms, apart from some itching, but they were disturbed by the cosmetic appearance. All four patients were examined by the departments of internal medicine, of ophthalmology, of ear, nose and throat diseases and, in the case of the women, by the department of gynaecology. The sternal marrow was normal, with the exception of a small increase of morphologically normal plasma cells in patient 1. No sign of leukemia or reticulosis was found. Chest X-rays were normal in all four patients. All patients were exposed to the full spectrum of xenon light without pathological reaction. The syphilis serology was negative; blood count and urine were normal, the ESR slightly elevated or normal. There were no uro- or erythroporphyrins in the urine. LE-cells were negative, as was the LE-latex test. Electrophoresis and other laboratory reactions were normal. Patch and scratch tests with the drugs and cosmetics used by the patients remained negative. The dermatosis was resistant to all external and internal therapy, including antihistamines and corticosteroids, and disappeared only when the patients were given Rhetis (chloroquine diphosphate 65 mg, hydroxychloroquine sulphate 50 mg, mepacrine hydrochloride 25 mg). This treatment was suggested by Dr Ute Linker. No other antimalarials were given.

CASE REPORTS

Case 1

G.K., a 31-year-old female clerk. Two sisters and her mother suffered from diabetes mellitus. In 1968 she was operated on for nephrolithiasis. In 1964 the dermatosis was first seen as a small red macule between the breasts. Afterwards the erythema spread slowly towards the submammary region, especially on the left side and onto the left upper abdomen. The middle of the upper back was also affected. In 1969 the condition worsened after intensive sunbathing on holiday in Spain. All forms of treatment proved unsuccessful, and the patient was sent to our outpatients department in 1970. She did not complain of itching or burning. She had occasionally been taking a common analgesic, saline laxatives, and oral contraceptives, which were prescribed from 1968 to 1969.

Examination. The patient was in good condition, apart from an adnexitis, diagnosed by the department of gynaecology.

The skin of the chest was affected beneath the clavicles (on the left side more than on the right), on and between the breasts, on the upper left abdomen (Fig. 1), and on the middle of the upper back (Fig. 2), by a sheet-like reticular reddish-blue, sometimes confluent, erythema of variable intensity. A slight infiltration was present in the centre of the erythema. The borders of the erythema were not always clearly defined, and in places there were thin, tongue-like spurs.

Histology. A biopsy from the left upper abdomen showed an epidermis which was thin, with short and sometimes broad epidermal ridges. A perivascular mantle-like round-cell infiltrate was found throughout the entire dermis. This infiltrate was more pronounced in the deeper corium and sometimes around the hair follicles, especially near the epidermis. The nuclei of the round cells were small, regular in form and very dark in colour. Within the infiltrate there were argyrophilic fibres (Gomoristain). The elastic fibres were fragmented. The blood vessels were dilated, and the connective tissue revealed a slight oedema. With Giemsa-staining only a few mast cells were seen, and with the alcian blue–PAS-technique, the PAS-positive membrane beneath the epidermis was found to be normal. In the upper part and middle of the dermis, there were abundant deposits of a substance which stained with alcian-blue, but was not stained by mucicarmine and was not metachromatic in the Giemsa-stain. The deposits were localized between the collagenous bundles as a partially homogeneous and partially granular material.

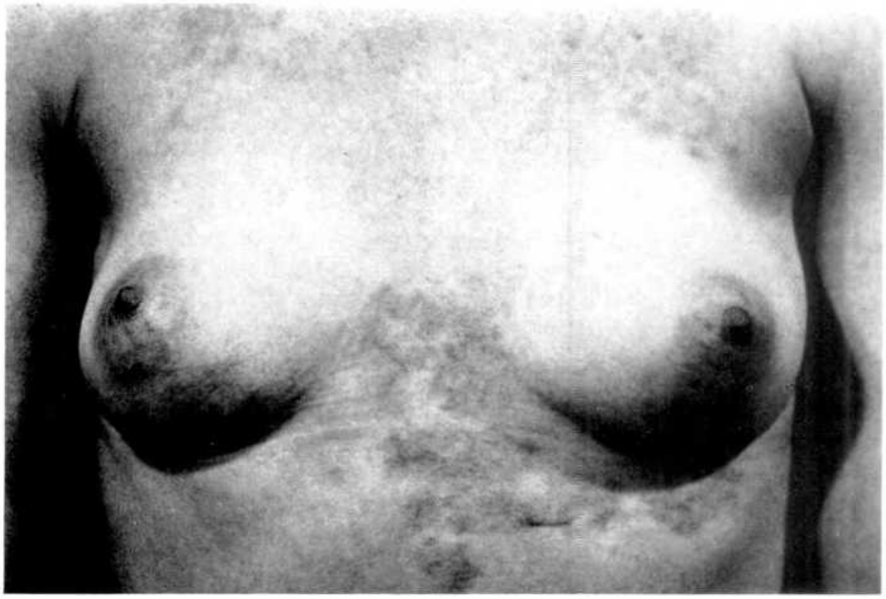

FIGURE 1. Patient 1. Sheet-like, reticular, reddish-blue, sometimes confluent erythema of variable intensity beneath the clavicles, between and on the distal parts of the breasts, and on the left upper abdomen.

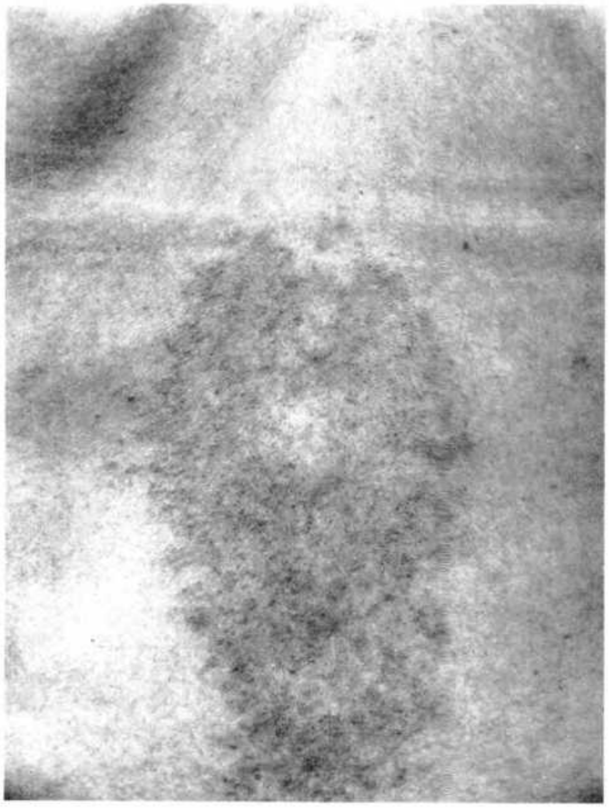

FIGURE 2. Patient 1. Net-like erythema in the middle of the upper back.

A biopsy of the back revealed similar findings to those in the biopsy from the left upper abdomen, except that a more dense perivascular infiltrate was present in the upper part and middle of the dermis.

Laboratory findings. There was a suspicion of latentd iabetes (blood sugar 2 h after 50 g glucose taken orally: 183 mg%).

Treatment. The patient was given three tablets of Rhetis daily, from 25 April 1972. By 25 May the erythema had considerably improved, and the dose was lowered to two tablets daily. When the dose was further reduced to one tablet daily, the erythema returned. The dose was therefore raised again to two tablets daily. By 29 September the erythema had completely disappeared. Therefore on 1 November 1972, Rhetis was discontinued, but the erythema returned on 4 December 1972. It disappeared again after treatment with Rhetis.

Case 2

I.K., a 20-year-old female student. Her maternal grandmother had diabetes mellitus. In 1966 the patient underwent a tonsillectomy, but had had no serious illness. In summer 1966 she noticed the first changes in the skin of that part of the breasts which was not covered by her swimsuit. Afterwards the dermatosis slowly spread towards the neck, to the skin between the breasts and to the middle of the upper abdomen. The condition worsened after sun bathing. She had been taking oral contraceptives since 1969, and 'rarely' took analgesics. She used only the usual products for personal hygiene and cosmetic purposes. The condition had been treated with corticosteroid creams and other ointments, but without success. In 1970, she was sent to our outpatients department.

Examination. The skin of the upper chest, on and between the breasts, and on the upper and middle part of the abdomen, was covered by a reticular reddish-blue, sometimes confluent erythema, with a slight infiltration in the centre. The borders of the erythema were not always clearly defined, and in places there were thin, tongue-like spurs.

Histology. Biopsies from the intermammary region and from the left breast (Fig. 3) revealed findings similar to those already described. In the first biopsy, throughout the dermis there were abundant deposits of a substance staining with alcian blue between the collagenous bundles, and in the second biopsy, there were numerous deposits in the upper and middle part of the dermis. After exposure to hyaluronidase (Merck, Darmstadt, 250 USPE/mg, 0·1 g, NaCl 0·16 g, 0·1 M phosphate-buffer pH 5·8 20 ml), the substance remained unstained with alcian blue, but there was not enough tissue left to repeat the procedure.

In a section of the fifth case, mentioned at the beginning, we obtained similar results after digestion with hyaluronidase.

Treatment. The patient was treated as from 25 April 1972, with three tablets of Rhetis daily. After 1 week she was given two tablets daily, and after 2 weeks of treatment a distinct improvement was noted. The dosage was lowered accordingly to one tablet daily, but the dermatosis returned. The patient was again treated with three tablets, and later with two tablets daily. After all signs had disappeared, she received one tablet daily. By 29 September 1972, there had been no recurrence despite a considerable degree of sunburn. On 1 November 1972, the treatment was discontinued, and she remained free of signs.

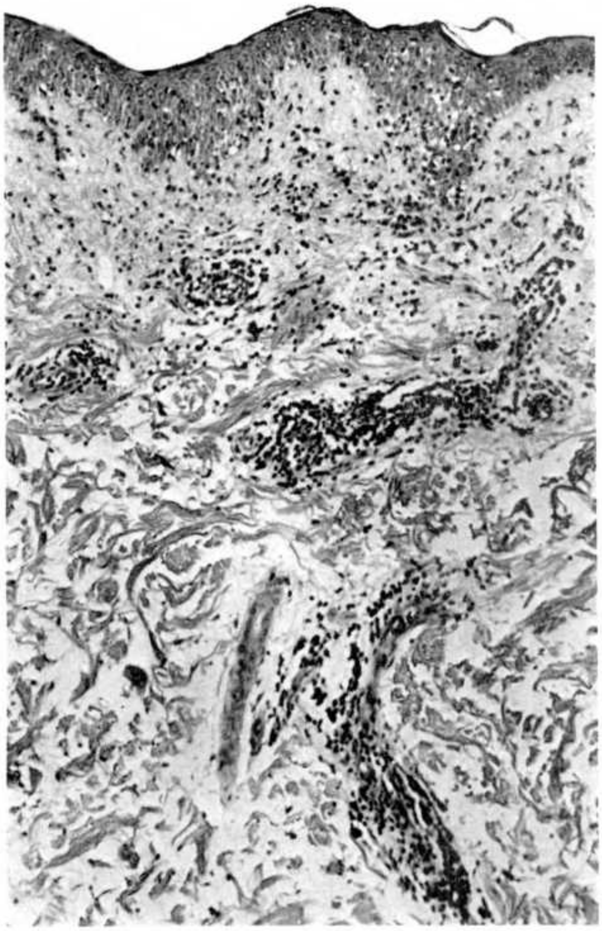

FIGURE 3. Patient 2. Slight spongiosis of the epidermis. Perivascular round cell infiltrate of varying intensity. A few round cells from the upper corium permeate the epidermis (Giemsa, × 100).

Case 3

M.B., a 39-year-old male clerk (1971). His mother died of leukemia. As a child he had had scarlet fever, mumps and measles. In the summer of 1960, the dermatosis started with red macules on the chest and on the back. It improved in winter, but otherwise spread slowly. The patient rarely took analgesics; he used only the usual products for personal hygiene and cosmetic purposes. He did not complain of itching, even after exposure to the sun. He was treated by a general practitioner and a dermatologist with corticosteroid and other ointments, but without success. In 1971, he was sent to our outpatients department.

Examination. In the middle of the chest and on the back there was a pink net-like erythema with small tongue-like spurs in the border zone. The borders of the erythema were relatively clearly defined, and the centre was slightly infiltrated. In the erythema there were some small teleangiectases (Fig. 4).

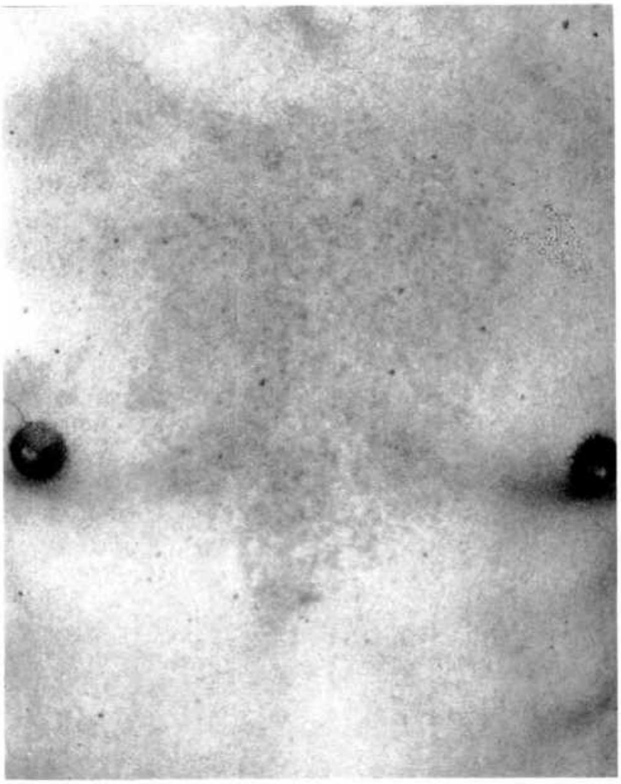

FIGURE 4. Patient 3. In the middle of the chest there is a net-like, pink erythema with small tongue-like spurs at the edges.

Histology. The chest revealed similar findings to those described in the biopsies from the other patients. In the upper, middle, and also to some extent in the deeper part of the dermis, there were numerous small areas of perivascular round cell infiltrate.

Treatment. Beginning on 25 April 1972, the patient received one tablet of Rhetis three times a day, and after 8 days some improvement had already been noted. The dose was therefore reduced to two tablets daily. By 13 May 1972, the dermatosis had nearly disappeared. During the summer vacation the patient received one tablet of Rhetis a day and was protected by a sun cream. There was no recurrence of the signs. From 30 September 1972, he was given half a tablet of Rhetis daily and on 31 October 1972 the therapy was discontinued with no recurrence so far.

Case 4

I.L., a 31-year-old housewife. The patient had measles as a child, a tonsillectomy in 1952 and an appendicectomy in 1956. In 1970 she was treated with 100 tablets of Novothyral [(L-3, 5, 3′-triiodothyronine (T_3), sodiumsalt 20 μg, L-3, 5, 3′, 5′-tetraiodothyronine (T_4), sodiumsalt, L-thyroxine, sodium-salt 100 μg)] for a diffuse goitre with euthyroidism. At the beginning of 1971 the dermatosis started with two red macules on the upper chest without prior exposure to the sun. During the summer vacation of 1971, she complained of itching after sun exposure. The skin changes became more intense and started spreading. Corticosteroid creams were applied, and also prednisone tablets taken, but without success; in fact, more and more of the skin became affected. In 1972, she was sent to our outpatients department. She had been taking Progylut (11 dragées ethinyl-oestradiol 0·05 mg, one daily; then 10 dragées ethinyloestradiol 0·05 mg, 17α-ethinyl-19-nortestosterone acetate 2 mg, one daily), occasionally Oxazepam and vegetable laxative tablets. She used the usual products for personal hygiene and cosmetic purposes.

Examination. The patient had a more or less intense, net-like erythema without sharp borders but with small tongue-like spurs. It was located on the upper chest beneath the clavicles, spreading from there to the intermammary region, and also, to a lesser degree, between the shoulder blades. The erythema was bluish-red on the breast and pink on the back. The centre revealed slight infiltration. Painless lymph nodes of pea size were present in the left axilla and in both inguinal regions.

Histology. The upper chest revealed findings similar to those in the other patients.

Treatment. From 17 February 1972, she was treated with three tablets of Rhetis a day, and after 10 days with two tablets. By 16 March 1972, the skin changes had disappeared, and treatment was continued with one tablet daily. During the summer, a sun cream and half a tablet of Rhetis were prescribed. In spite of sun bathing, the patient remained free of signs and symptoms. On 8 October 1973, the therapy had to be discontinued because of a reduction in visual acuity and pathological electroretinogram and electro-oculogram findings by the ophthalmological department. By 16 November 1972, her visual acuity had returned to normal. Since then she has been without signs and symptoms.

DISCUSSION

All four patients revealed similar signs and symptoms.

(1) Skin manifestations began in the breast area, spreading to the upper abdomen and the middle of the back. Some areas of the skin exposed to the sun, such as the face and the back of the hands, were never affected. No atrophy, follicular hyperkeatosis or parakeratosis were ever observed.

(2) All the patients had net-like, sheet-like, reddish-blue erythemas with small tongue-like spurs at the border and slight central infiltration. The border 1, 2, 4 was not always clearly defined (Figs. 1–3).

(3) All the patients revealed perivascular infiltrates of round cells (Fig. 5) and deposits of an alcian blue-staining substance in the dermis, possibly acid mucopolysaccharides. The material was not metachromatic and remained unstained after digestion with hyaluronidase, as far as we could tell from our preliminary results with this technique. The deposits did not stain with mucicarmine. No stellate shaped cells were present, and the collagenous bundles were preserved. On the other hand, the alcian blue-staining material was different in quantity and quality from the appearance of similar staining substances in inflammatory processes. Steigleder called this latter phenomenon 'phanerosis' (Steigleder, 1959; Steigleder & Weakley, 1961). The round cells consisted predominantly of lymphocytes. Very few plasma cells and a few larger cells were present, suggestive of stimulated lymphocytes (Steigleder 1972). Unfortunately the patients did not agree to more and larger biopsies in order to classify the cells and deposits by histochemical methods. No atrophy of the epidermis and no changes in the PAS-positive membrane were to be found (Figs. 3 and 5).

(4) With one exception the skin changes took several years to spread.

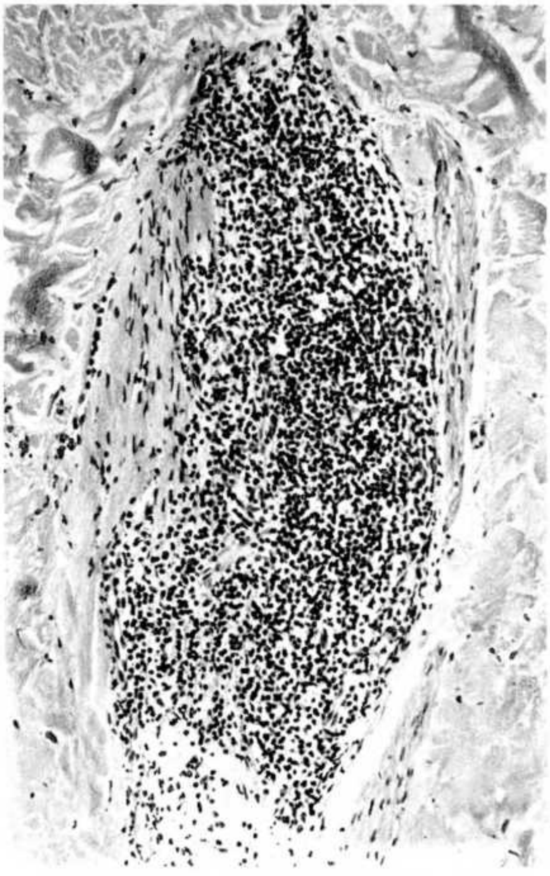

FIGURE 5. Round cell infiltrate (patient 1) (H & E, × 250).

(5) In two patients the skin changes began after sun exposure, and worsened after intense exposure to sun.

(6) The disease could not be influenced by giving corticosteroids locally or in one case systemically, but it improved after oral administration of Rhetis.

There is no proof of any allergy. The possibility of a photo-dermatosis was considered but exposure to the full spectrum of xenon light revealed normal reactions in all four patients, and the eruption did not resemble any photodermatosis known to us or to Professor I. Magnus (personal communication). There is a similarity to the plaque-like form of cutaneous mucinosis described by Perry, Kierland & Montgomery (1960), but the clinical picture differs slightly and the histology and histochemical findings are different. In contrast to our cases of REM syndrome, in their cases the alcian blue deposits were metachromatic, stained with the PAS-reaction and with mucicarmine, and were 'most heavily deposited beneath the epidermis' and 'distributed in a patchy manner'. The American authors mentioned that the deposits were eradicated by pretreatment with hyaluronidase. As only one section was treated and no further data were given about this procedure, it is difficult to compare this reaction with our results. As already mentioned, we had difficulties with this technique in REM syndrome, in contrast to other skin conditions we had investigated previously. Remarkably, the lesions of one of the

American patients disappeared for 6 months after treatment with an antimalarial drug, but 'the subsequent follow-up indicated that such therapy was without value'. We have already mentioned the difference between the deposits in REM syndrome and the appearance of alcian blue-staining material in inflammatory processes. Perry *et al.* (1960) believed that their patients were suffering from lichen myxoedematosus, and according to the histology of their patients they may have been right. It was also suggested to us that REM syndrome is a variety of seborrhoeic dermatitis. Unfortunately, we have not seen this condition in Cologne. Patients referred to us with this diagnosis have in fact been diagnosed as suffering from contact dermatitis, drug eruptions, mild or atypical psoriasis or mycoses and related conditions. The histological features of seborrhoeic dermatitis, especially acanthosis and parakeratosis, as described by Pinkus & Mehregan (1969) were absent in REM syndrome. Lupus erythematosus may look similar to REM syndrome, but additional signs are to be found, and changes of the PAS-positive membrane beneath the epidermis, follicular hyperkeratosis and epidermal atrophy are absent. The distribution pattern of lupus erythematosus is different from REM syndrome. Our laboratory tests were not consistent with lupus erythematosus.

In conclusion, therefore, we were not able to relate the clinical and histological pictures to any known dermatological entity.

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ADDENDUM

Very recently we have observed two young women with the macroscopic and microscopic signs of the plaque-like form of cutaneous mucinosis (Perry *et al.*, 1960), which is very similar indeed to the REM syndrome. One patient was cleared by treatment with Rhetis after 6 years of unsuccessful therapy; in the other, treatment with Rhetis has just started.

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An investigation of the skin flora of dialysis and transplant patients

W.C.NOBLE, M.H.REBEL AND INGRID SMITH

Department of Bacteriology, St John's Hospital for Diseases of the Skin, London, E9 6BX,
and Hospital Epidemiology Unit, University Hospital, Leiden, The Netherlands

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SUMMARY

The skin flora of patients on routine haemodialysis and of outpatients with a renal transplant has been investigated and compared with that of rheumatology outpatients at the same hospital. Dialysis patients had significantly smaller numbers of organisms on the skin. No excess of pathogens or potential pathogens was found in any group.

Patients in renal failure may have unusually severe desquamation of the skin (Giordano *et al.*, 1972). Patients with urea retention have twice the quantity of nitrogenous compounds on the skin compared with normals and skin surface sugar concentrations are 50% above normal (Bollinger & Gross, 1956). Similar changes, though of less magnitude, might be expected on the skin of patients who undergo routine haemodialysis on a twice-weekly basis although the blood urea levels are much lower than in patients with urea retention. Dialysis patients may experience drying of the skin, perhaps due to water withdrawal (Public Health Laboratory Service, 1968). Such changes might nevertheless be expected to bring about ecological shifts in skin micro-organisms and such shifts might create a reservoir of potential pathogens from which infections might arise. Patients on routine haemodialysis frequently suffer shunt infection with *Staphylococcus aureus* (Public Health Laboratory Service, 1968) and patients with a renal homotransplant may be infected with a variety of micro-organisms (Rifkind *et al.*, 1964, 1967; Myerowitz, Medeiros & O'Brien, 1972). Skin infections may contribute to the death of transplant patients (Haim *et al.*, 1973).

It therefore seemed worth investigating the skin flora of patients with a renal transplant or on haemodialysis to see what changes had taken place. Rheumatology patients of the same sex and approximately the same age were chosen as controls, they had a similar outpatient attendance rate, though they were not generally receiving antibiotics or immunosuppressive drugs.

MATERIALS AND METHODS

Patients attending twice-weekly at the dialysis unit of the University Hospital in Leiden were swabbed at a routine dialysis session. Transplant and rheumatology patients were those attending normal outpatient clinics for a routine check-up and were sampled as they became available. A total of twenty-three dialysis, twenty-three rheumatology and twenty-one transplant patients were available for sampling. Transplant patients were well maintained and had mostly received their transplants long before sampling; one patient had received the transplant 2 months and five, 4 to 12 months previously. Seven had received transplants between 1 and 3 years and seven between 3 and 6 years previously; in one instance the transplant had been carried out in another country. Each patient entering the investigation was sampled, using cotton tipped swabs moistened with broth, at the following sites chosen to represent both known carrier sites and 'normal' skin surfaces; nose, gums, chest, perineum, toewebs, axilla and lower leg (dialysis patients were sampled on the forearm rather than the leg if this was the site of the shunt).

Swabs were inoculated immediately on to blood agar (aerobic and anaerobic incubation), CLED (a non-inhibitory diagnostic agar which inhibits the swarming of Proteus species), McConkey and two Sabouraud plates (pH 5) for qualitative analysis. In addition, contact plates were made of the chest, thigh and forearm on serum agar. All plates except those containing Sabouraud's agar were incubated at 37°C and examined at 18 and 36 h. One Sabouraud plate was incubated at 37°C for 1 week and the other at ambient temperature (20°C) for 2 weeks or longer. Quantitative counts were made on the contact plates. All organisms from the qualitative plates were identified at least to the generic level. Coagulase negative, Gram positive cocci were grouped according to the scheme of Baird-Parker (1965) and diphtheroid organisms according to the scheme of Somerville (1973).

RESULTS

Qualitative results obtained by swabbing

Tables 1–3 show the results obtained from these patients. It is clear that there were no major differences between the groups of patients and that their flora did not differ markedly from that expected from normal healthy people (Noble & Somerville, 1974). When the results were examined for a total species count, however, it became evident that dialysis patients had a more restricted flora than the other groups (Table 4).

TABLE 1. Carriage of normal skin flora

	Coagulase negative cocci*						Diphtheroids†		
	Staphylococci			Micrococci					
	Rh	Di	Tr	Rh	Di	Tr	Rh	Di	Tr
Nose	39	37	31	3	4	4	29	29	22
Gums	2	1	1	3	0	1	23	15	27
Chest	37	45	36	18	17	19	18	26	30
Perineum	42	36	35	15	12	18	46	29	30
Toewebs	38	37	31	20	26	25	45	28	30
Axilla	33	35	23	10	10	12	28	16	26
Leg/arm	40	16	28	31	4	22	37	14	33

* Number of distinct strains isolated according to the scheme of Baird-Parker (1965). The number of strains carried by each patient has been summed.

† Number of distinct strains isolated according to the scheme of Somerville (1973). The number of strains carried by each patient has been summed.

Rh, rheumatology (twenty-three patients); Di, dialysis (twenty-three patients): Tr, transplant patients (twenty-one patients).

TABLE 2. Carriage of opportunist or potential pathogens

	Candida albicans			Other yeasts*			*Staph. aureus*			Enterococci†			Gram negative rods‡		
	Rh	Di	Tr	Rh	Di	Tr	Rh	Di	Tr	Rh	Di	Tr	Rh	Di	Tr
Nose	0	0	1	0	0	0	12	13	8	0	1	0	9	6	6
Gums	6	6	9	4	3	2	0	1	2	1	0	0	2	5	4
Chest	0	0	0	0	1	0	5	4	4	1	1	0	5	2	5
Perineum	1	1	4	1	0	1	1	4	1	3	2	2	4	6	8
Toewebs	2	0	1	0	3	2	0	2	2	1	1	0	2	4	1
Axilla	0	0	1	0	1	0	1	4	1	0	0	0	4	3	9
Leg/arm	0	0	0	0	0	0	4	3	3	2	1	1	8	2	2

* In one instance only were filamentous fungi found; one rheumatology patient yielded *Trichophyton mentagrophytes* from the toewebs.

† Beta haemolytic streptococci other than Group D were isolated on two occasions only.

‡ Including *Escherichia coli*, Citrobacter, Mima, Enterobacter, Klebsiella, *Proteus mirabilis* and *Proteus morgani*. Rh, rheumatology; Di, dialysis; Tr, transplant patients.

TABLE 3. Carriage of other micro-organisms

	Alpha and gamma* Streptococci			Neisseria		
	Rh	Di	Tr	Rh	Di	Tr
Nose	3	7	1	3	1	2
Gums	77	42	52	18	25	20
Chest	3	6	6	1	1	0
Perineum	2	1	1	0	0	0
Toewebs	4	0	0	0	0	2
Axilla	1	0	0	0	0	1
Leg/arm	7	0	0	1	1	0

* Strain divided according to colony morphology and haemolysis only. Rh, rheumatology; Di, dialysis; Tr, transplant patients.

TABLE 4. Pooled results for microbial species. Results expressed as a percentage of total strains carried by rheumatology patients

	Rh	Tr*	Di
Nose	100	84	100
Gums	100	95	72
Chest	100	124	117
Perineum	100	95	79
Toewebs	100	92	90
Axilla	100	104	90
Leg/arm	100	75	31
Mean count	100	94	79
Total patients	23	21	23

* Results corrected for number of patients.

Quantitative results obtained from contact plates

Although the differences between the groups at each site are not large, and only reach statistical significance on the thigh, dialysis patients are consistent in having fewer colony forming units on their skin than the other two groups (Table 5). If the counts from all sites are pooled, dialysis patients have significantly fewer colony forming units of bacteria on their skin ($P<0.1\%$).

The geometric mean count was used throughout this series as this accords better with the distribution of counts in normals (unpublished observations).

Therapy

Table 6 shows the therapy received at the time of sampling. The antibiotics were entirely synthetic penicillins (Cloxacillin and Ampicillin). Prednisone and azathioprine were exclusive to the transplant patients (one rheumatology patient received a parenteral cortisone compound). 'Other' therapy was

TABLE 5. Contact plate counts

Site		Geometric mean count per cm^2	Analysis of variance
Chest	Rh	11·7	NS
	Tr	14·4	
	Di	5·5	
Forearm	Rh	7·3	NS
	Tr	6·5	
	Di	4·5	
Thigh	Rh	14·1	$5\% > P > 1\%$
	Tr	22·4	
	Di	7·1	
Total	Rh	10·7	$P < 0{\cdot}1\%$
	Tr	19·9	
	Di	5·7	

Rh, rheumatology (twenty-three patients); Tr, transplant (twenty-one patients); Di, dialysis (twenty-three patients). NS, not significant.

that appropriate to the group, antidepressant drugs in the transplant group, anticoagulants for the dialysis group and aspirin compounds for rheumatology patients.

DISCUSSION

Although many minor changes in the skin flora under various conditions of health or disease have been described, major changes in composition appear to occur only when hydration is increased by occlusion (Marples, 1965) or in terminal illness (Stratford *et al.*, 1968; Gallus, Stratford & Dixson, 1969). In the present investigations, dialysis patients proved to carry fewer bacteria, both qualitatively and quantitatively, than either rheumatology or transplant patients, though the reverse was expected. This difference is presumably a reflection of the drier skin of these patients, since adults in the seventh decade and older also have a drier skin and a simpler skin flora than those who are younger (Somer-

TABLE 6. Therapy received at time of sample

	Rh	Tr	Di
Antibiotic	0	6	3
Corticosteroid	1	21	0
Cytostatic	0	21	0
'Other'	12	16	23
Total patients	23	21	23

Rh, rheumatology (twenty-three patients); Tr, transplant (twenty-one patients); Di, dialysis (twenty-three patients).

ville, 1969). By analogy with patients with urea retention, haemodialysis patients might be expected to excrete larger quantities of nitrogenous compounds and sugar onto the skin surface. Although this *per se* might lead to changes in the composition of the skin flora, it seems probable that desiccation is a more important factor.

Renal transplant patients may become infected with a variety of common and uncommon pathogens or even 'commensals' and such infections may contribute towards death. It is presumed that the prescription of cytostatic agents and corticosteroids, necessary for the maintenance of the graft, predispose the patient to infection. Major episodes of infection in transplant patients are associated with graft rejection and the degree of chemotherapeutic interference (Bach *et al.*, 1973; Bell & Briggs, 1973; Burton & Greenough, 1973). The organisms involved are frequently members of the faecal flora, although some workers report infection with fungi which are unlikely to be of gut origin. Although Rifkind *et al.* (1964; 1967) found that nasal or skin carriage of *Staph. aureus* was likely to lead to infection with this organism, they could not find sources for the other infecting organisms. Haim *et al.* (1973) have reported thirty-nine instances of skin infection in thirty-five kidney transplant patients; ten were pyogenic and twenty-one were fungal infections, the remainder being of viral aetiology. No attempt to find the source of the organisms was described.

There is no evidence from the present series of investigations that the skin of dialysis or transplant patients, who are well maintained, harbours organisms other than those found in normal healthy individuals. All the patients in this series were living at home and leading normal lives within the limits prescribed by their disease; their skin floras seem to reflect this normality.

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Case Reports

Acute generalized pustular bacterid

AN UNUSUAL MANIFESTATION OF LEUKOCYTOCLASTIC VASCULITIS

R.S.-H.TAN

Department of Dermatology, Westminster Hospital, London, S.W.1

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SUMMARY

A woman, aged 58 years, suddenly developed a widespread sterile pustular and purpuric eruption after a streptococcal infection. The eruption resolved spontaneously within a few weeks. Histopathology showed an intra-epidermal pustule with underlying leukocytoclastic vasculitis. The cutaneous vasculitis in this patient presented as a true pustular bacterid.

Most cases of sterile pustular eruption are considered to be manifestations of psoriasis, whether localized to the extremities as in acropustulosis (Baker & Wilkinson, 1972), or more widespread as in generalized pustular psoriasis (Baker & Ryan, 1968). Persistent pustular eruption of the palms and soles is also considered to be of the same nature (Barber, 1930; Ingram, 1930; Enfors & Molin, 1971), but Ashurst (1964) and Hellgren & Mobacken (1971) thought otherwise. Andrews & Machacek (1935) implicated a focus of infection, calling their cases pustular bacterid; but Everall (1957) found that the removal of the septic focus did not cure the eruption. The striking feature of palmo-plantar pustulosis is its chronic course whether or not psoriasis is present (Enfors & Molin, 1971) or a septic focus is eradicated (Hellgren & Mobacken, 1971). Sneddon & Wilkinson (1956) described a distinct group of sterile pustular eruptions, involving the trunk and responding to dapsone (4,4′-diaminodiphenylsulphone), which they distinguished from pustular psoriasis; Hellier (1956), however, tried to relate this group to Andrews' pustular bacterid.

This report describes a patient with an acute sterile pustular eruption with features that would support the concept of a true generalized pustular bacterid.

CASE REPORT

A woman, aged 58 years, suddenly developed a pustular eruption on the dorsa of both hands. Within a day, this eruption had extended to involve her limbs and to a lesser extent her neck and trunk. She had developed a sore throat associated with malaise and fever 4 days earlier, for which she had taken one tablet of penicillin. There was no previous or family history of skin diseases.

On examination

A scattered symmetrical pustular rash affected mainly the dorsa of both hands (Fig. 1), her forearms, legs (Fig. 2), and to a lesser extent her neck and trunk. The pustules were of varying sizes up to 6 mm in diameter. Each pustule was discrete, surrounded by a narrow rim of erythema and set upon normal skin. On the surface of many was a central elevation which was probably a follicular opening. There were also a few purpuric lesions on the legs (Fig. 2). The mouth, nails and body flexures were not involved. There was no lymphadenopathy and general medical examination was normal.

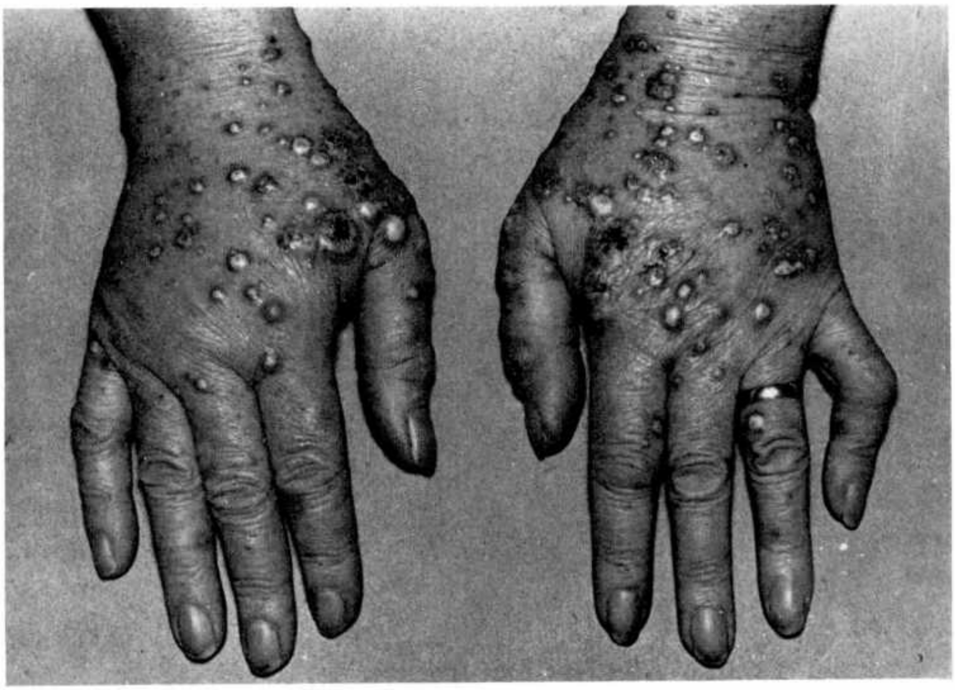

FIGURE 1. Discrete pustules on dorsum of hands. Many seem to be centred on hair follicles.

Most of the pustules rapidly resolved in 8–10 days by drying up into brownish papules. Over the following weeks some of the papules on the dorsa of the hands enlarged, the follicular openings became patulous and milia appeared. On the lower legs, the lesions became more haemorrhagic; necrotic crusted areas developed which healed slowly.

For the first 3 weeks after hospital admission, fresh pustules continued to erupt. These were similar to the original lesions but were smaller, more widely scattered, and much fewer in number; pustules appeared on the palms, soles and lips. During this period her temperature fluctuated around 37·2°C; this returned to normal 2 days after the appearance of the last pustule.

A course of erythromycin was given 4 weeks after admission. At this time some pustules were still present although they were probably resolving. It is unlikely that this course of antibiotics affected the natural history of the disease. One tablet of penicillin was also given; this did not reactivate the condition. By the time the patient left hospital the majority of the lesions had resolved. There has been no recurrence after 1 year.

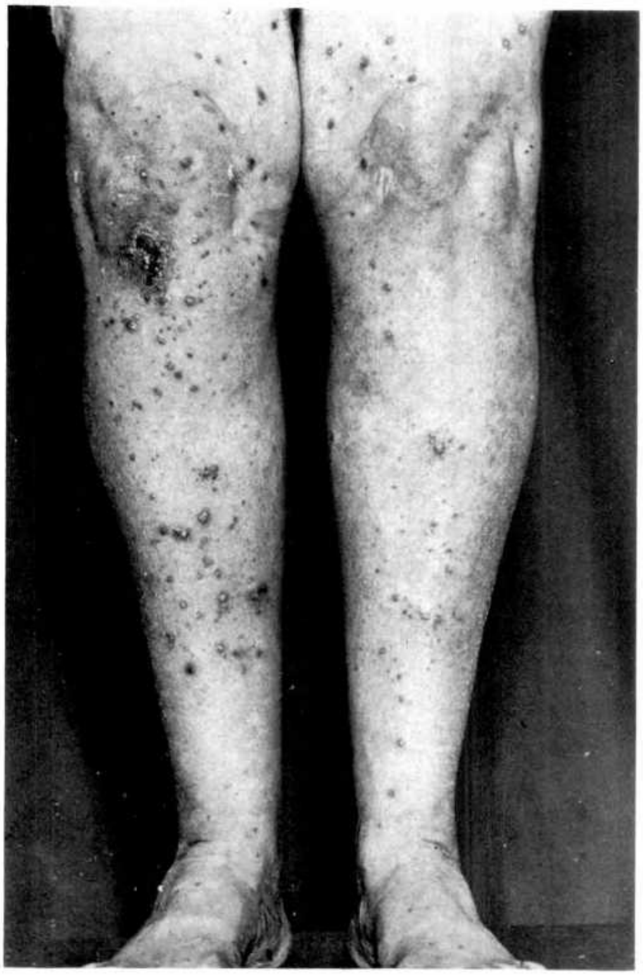

FIGURE 2. Pustular and purpuric lesions on legs.

Investigations

White blood count (on admission) 14,300/mm^3; 86% polymorphs, 8% lymphocytes. Platelets normal. ESR 10 mm in 1 h.

Repeated cultures from the pustules were either sterile or produced a scanty growth of coagulase negative staphylococci. Blood cultures, throat swabs, urine cultures and high vaginal swab were all negative.

Antistreptolysin O titre (ASO titre): day 6, 800 Todd units; 2 weeks, 1200 Todd units; 3 weeks, >1600 Todd units; 5 weeks, 1200 Todd units; 7 weeks, 1200 Todd units.

Anti-DNAse B titre: 3 weeks, 3200 units (upper normal 250 units).

Serum proteins: albumin 3·2 g/100 ml; globulin 5 g/100 ml. Protein electrophoresis, raised α_2 and γ globulins.

Serum immunoglobulins: IgG, 1900 mg/100 ml (normal 500–1600 mg/ml); IgA, 440 mg/100 ml (normal 125–425 mg/100 ml); IgM, 78 mg/100 ml (normal 47–170 mg/100 ml).

Auto-antibody screen. Positive at 1 in 10, thyroid microsomes: negative; thyroid colloid, gastric parietal cells, mitrochondria, smooth muscle, anti-nuclear factor. Latex RA negative.

Normal or negative results: culture of skin for acid fast bacilli; viral studies (including mycoplasma); mycological studies; X-rays of chest, sinuses and middle ear; haemoglobin; blood glucose; urea; electrolytes; calcium; phosphate; bromide; liver function tests; Wassermann reaction; gonococcal complement fixation test.

Histopathology (Dr I. Whimster)

In a 5–6-day-old pustule (Fig. 3): there is an intra-epidermal collection of polymorphonuclear leukocytes with surrounding epidermal oedema. A few red blood corpuscles are present in the epidermis. Immediately under the pustule is an area of leukocytoclastic vasculitis, around which is a chronic inflammatory cell infiltrate. There is no evidence of psoriasis. Special stains do not show the presence of micro-organisms.

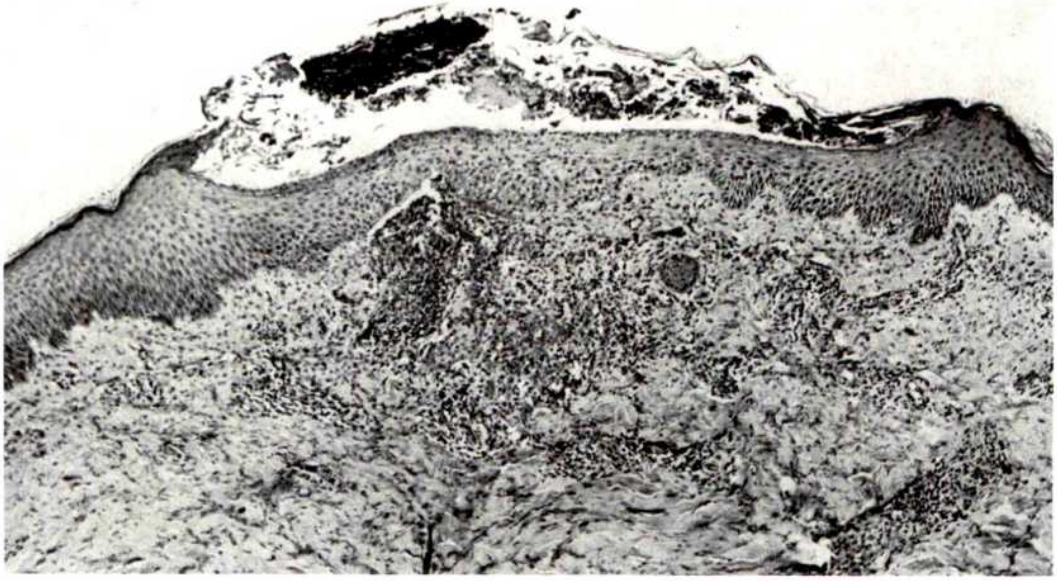

FIGURE 3. Intra-epidermal pustule with underlying leukocytoclastic vasculitis (× 16).

Three-week-old lesion on dorsum of hand: there are milia or keratin-filled cysts set in a densely fibrotic superficial dermis, which is separated from the deep dermis by chronic inflammatory cells including numerous foreign body type giant cells.

Reactions to bacterial antigens

(1) 0·2 ml. of Bencard's bacterial antigenic suspensions F1–5 and a control were injected intradermally into the patient's fore-arm. There was no significant reaction when compared with the control at 15 min or 6–8 h. From 48 h onwards the F1 site became markedly inflamed, indurated and a pustule developed; this eventually discharged on day 7.

(2) 0·04 ml of the individual components of Bencard's F1 group (*Strepococcus pneumoniae*, Strep. Group C, Strep. Group G, *Strepococcus salivarius*) and a control were injected intradermally into the other fore-arm. There was no significant reaction when compared with the control at 15 min or 6–8 h. From 48 h onwards erythema and induration were present at the site of Strep. Group C. This reached its maximum at 72 h (2 cm induration) but never became pustular.

(3) Heaf test—positive.

DISCUSSION

Wilkinson (1969) classified pustular dermatoses anatomically according to the site in the skin of neutrophil aggregation. This occurred mainly in the subcorneal region in psoriasis and in subcorneal pustular dermatosis. Furthermore, he described a secondary type in which the subcorneal pustules were due to deeper abscesses breaking through into the epidermis. He had seen this in acute allergic vasculitis. It is conceivable that the same process occurred in this patient and that this case represents a true pustular bacterid.

The following features are in favour of such a concept:

(1) History of a sore throat, associated with a brisk and well marked antibody response to the streptococcus.

(2) Rapid resolution of the rash associated with a fall in the antibody titre.

(3) Mild pyrexia only during the eruptive phase.

(4) Lack of further pustule formation after the antibody response had reached its peak.

Various dermatoses, and in particular psoriasis, can be precipitated by factors such as a preceding haemolytic streptococcal sore throat, pregnancy or hypocalcaemia. However, the leukocytoclastic vasculitis underlying the pustule would favour a more specific hypersensitivity, perhaps of the Arthus type.

It seems that an acute generalized sterile pustular dermatosis such as in this case is rare. Hellgren & Mobacken (1971) mentioned an acute form of pustulosis palmaris et plantaris sometimes combined with pustules on the extremities and/or trunk which appeared after an upper respiratory tract infection and usually resolved rapidly. Baker & Ryan (1968) described five cases of acute exanthematic pustular psoriasis that also followed an upper respiratory tract infection. The rash usually started on the palms and soles, became generalized and then rapidly resolved with no subsequent recurrence. In none of their cases was there any evidence of psoriasis clinically nor in the previous or family history. The case described here resembles many of Baker & Ryan's (1968) cases. Their illustrative case, however, had histopathological features of psoriasis but it may not have been typical of the group as a whole as it was the only one with generalized erythroderma.

The results of intradermal testing with bacterial antigens are more difficult to interpret. A Group A streptococcus was likely to be involved in view of the marked anti-DNAse B response and the brisk rise in the ASO titre. A large dose was given in the first test because the bacterial test suspension used does not contain Group A Streptococci. Any reaction that might occur would be against antigens shared with other streptococcal groups; Streptococcus groups A, C and G do share certain antigens (Stewart, 1968). The lack of significant response at 6–8 h may reflect the absence of specific Group A streptococcal extract in this test suspension. The subsequent strongly positive delayed reaction with pustule formation could be the result of the large dose injected. Even so, it is significant that this response occurred only at the F1 site. The significant delayed reaction at the site of Group C streptococcus in the second test is probably the result of shared antigens with Group A (Stewart, 1968). A similar delayed reaction to streptococcal antigen has been demonstrated in two cases of erythema elevatum diutinum (Cream, Levene & Calnan, 1971). They did not get the same reaction with other vasculitides studied over the same period.

A non-allergic mechanism for pustule formation could result from streptococcal products, disseminated by the blood stream, lodging in the superficial dermal vessels and inducing a direct toxic effect on the neighbouring tissues. The damage produced could then attract neutrophils by a chemotactic mechanism (Spector, 1969). As no further pustules appeared after the ASO titre had reached its maximum value, it is possible a similar neutralizing antibody was induced against this 'toxic' streptococcal product. The rarity of the condition could be because only certain types of streptococci elaborate 'skin toxins', just as only certain types elaborate the erythrogenic toxin or are nephrotoxic.

This case has sufficiently distinctive features to separate it from other better recognized pustular eruptions, such as pustular psoriasis, subcorneal pustular dermatosis, and Andrews' pustular bacterid. This last condition cannot convincingly be separated from the main group of persistent palmo-plantar pustulosis. The use of the term bacterid in this chronic eruption only adds to the confusion and therefore should be discontinued.

Sneddon (1973) reported a case in which subcorneal pustules were associated with erythema multiforme following a probable viral upper respiratory tract infection; there was no leukocytoclasis on skin histopathology. Macmillan (1973) reported four cases of generalized pustular rashes attributed to drugs. The histopathology in each case was that of a dermal lympho-histiocytic infiltrate. Diaz, Provost & Tomasi (1973) described two patients with generalized pustular eruption; leukocytoclastic angiitis was present in the skin sections. The pustules subsequently ruptured leaving punched-out necrotic ulcers. The patients were acutely ill, had evidence of systemic involvement and both responded to large doses of systemic steroids. It could be that the case reported here is part of a spectrum of allergic sterile pustular dermatoses.

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Follicular mucinosis responding to dapsone

R.K.KUBBA* AND T.W.STEWART

Department of Dermatology, The University of Liverpool, Liverpool Royal Infirmary, Liverpool L3 5PU

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SUMMARY

A case report is presented of a patient with an eruption of acute onset shown to be follicular mucinosis by biopsy, and which responded to oral dapsone therapy.

INTRODUCTION

Pinkus (1957) first drew attention to mucinous degeneration of the hair follicles, when he described six cases under the title of 'alopecia mucinosa'. Jablonska, Chorzelski & Lancucki (1959) suggested 'follicular mucinosis' as a better term, since alopecia is not always apparent. The clinical picture is variable and diagnosis is dependent on the demonstration of mucin in the hair follicles. Over 150 cases have been reported, some of which have been associated with a reticulosis (Braun-Falco, 1957). We describe a case, the presentation of which mimicked pityriasis rosea and which responded to treatment with dapsone, a feature hitherto not reported.

CASE REPORT

A Caucasian male student, aged 19 years, was first seen on 20 October 1971 with a rash of 11 days' duration which had started as papules over the anterior abdominal wall and rapidly become generalized. He had suffered from severe pruritus and felt vaguely unwell for the first 3 days. He also had hay fever, and acne vulgaris. Previously he had had measles, chickenpox, mumps, tinea pedis and, until the age of 14 years, asthma. The family history was not significant and he had not been taking any drugs.

Examination revealed papules and plaques distributed along the cleavage lines on the trunk and limbs. Some of these lesions showed marginal scaling, and some had an eroded surface. Some of the plaques were slightly infiltrated. There were marked erythema, oozing, crusting and scaling affecting the entire face and scalp (Fig. 1). The palms, soles and dorsa of his hands and feet showed fine vesicles with some purpuric lesions on the dorsa of the feet. The mucosae were unaffected and there was no alopecia. Systemic examination showed no abnormality.

Investigations (21 *October* 1971)
Hb 15·0 g%; WCC 6600/mm^3; differential count: polymorphs 66%, monocytes 3%, eosinophils 15%, lymphocytes 16%; ESR 10 mm/h (Westergren); MSU sterile; plasma electrolyte levels,

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* Present address: Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

normal. Plasma protein levels; total 7·0 g%, albumin 4·1 g%, globulin 2·9 g%. Serum immunoglobulin levels; IgA 244 mg% (normal 90–450 mg%), IgG 1280 mg% (normal 800–1800 mg%), IgM 108 mg% (normal 60–250 mg%). Liver function tests; serum bilirubin 1·4 mg% (repeated on 26 October 1971, 0·8 mg%), serum alkaline phosphatase 8 KA units. Serum SGPT 29 mIU/ml. Screening test for glucose-6-phosphate dehydrogenase, normal. Chest X-ray, normal. Scrapings from left foot, no dermatophyte grown.

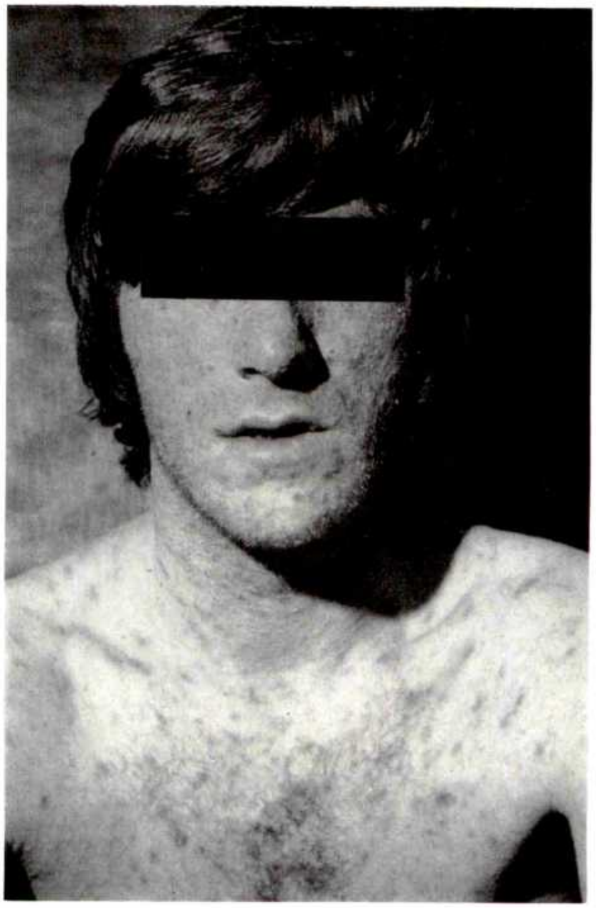

FIGURE 1. Lesions on face and upper chest.

A skin biopsy of a scaly papule from the right hypochondrium showed an acanthotic, parakeratotic and crusted epidermis, oedematous and displaying spongiotic vesicles. The upper dermis was markedly oedematous and there was a lymphoreticular infiltrate most marked around the blood vessels. There was a similar infiltrate containing some eosinophils around two hair follicles, which were grossly disrupted and showed degeneration. These changes were suggestive of follicular mucinosis, and this was confirmed by finding positive staining by alcian blue (Fig. 2).

Dapsone 100 mg/day was commenced on 25 October 1971. The skin lesions cleared dramatically and treatment was stopped 3 days later. The patient was discharged from hospital the following day.

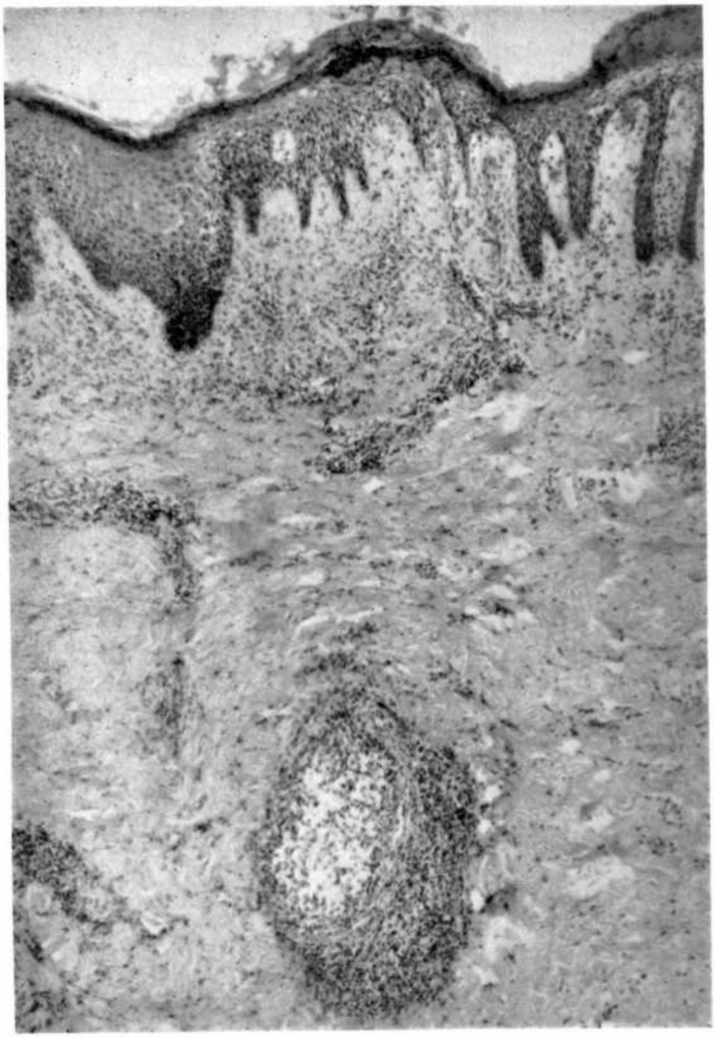

FIGURE 2. Biopsy showing epidermal and dermal changes including a disrupted hair follicle (H and E, ×42).

A short follow-up in the outpatient clinic and a subsequent communication (30 January 1973) confirmed a continued remission.

DISCUSSION

Follicular mucinosis is a curious dermatosis which characteristically presents as grouped follicular papules or slightly infiltrated plaques with patulous or plugged hair follicles (Emmerson, 1969). Alopecia further aids in clinical recognition. However, there are case reports of follicular mucinosis presenting as subcorneal blisters on an erythematous base (Vermenouze & Stephanopoli, 1963), acute and chronic folliculitis (Johnson, Higdon & Helwig, 1959), eczema and indurated annular lesions

(Emmerson, 1969, cases 30 and 39), and seborrhoeic dermatitis and neurodermatitis (Haber, 1961, cases 1 and 3). This patient had typical lesions of pityriasis rosea on the trunk, but also had involvement of the peripheral parts of the limbs, the face and the scalp, which does occur in pityriasis rosea but uncommonly (Rook, Wilkinson & Ebling, 1972). Nevertheless, because of possible alternative diagnoses, such as a pityriasiform seborrhoeid, a biopsy was performed. Between this being done and the report being available dapsone therapy was commenced, because of a description of its success in a case of vesicular pityriasis rosea (Russell Anderson, 1971) which it was felt this case most closely resembled. Only when the histology of a trunk lesion showed the classical changes of follicular mucinosis was the true diagnosis revealed.

This case further supports the belief shared by many that this disease is a non-specific reaction pattern (Haber, 1961; Hyman, 1962; Emmerson, 1969), and merely represents a structural change brought about by more than one aetiological agent. It is, therefore, tempting to suggest that some cases of apparent pityriasis rosea might have mucinous degeneration of the hair follicles which is not normally detected, as skin biopsy is usually not required for the diagnosis of this disease (Milne, 1972).

If a hair follicle is not included in the section, only the epidermal changes of spongiosis and vesiculation are seen, which occur in both pityriasis rosea and follicular mucinosis, and furthermore, in mild cases, an alcian blue stain is mandatory to make a definite diagnosis of follicular mucinosis.

Dapsone has been used with great success in several diseases but the response in this patient was remarkable. However, this must be viewed with a little circumspection since a large proportion of cases of follicular mucinosis show spontaneous resolution in a few weeks to a few months (Emmerson, 1969; Coskey & Mehregan, 1970). Nevertheless, the shortest reported duration of the rash is 4 weeks (Kim & Winkelmann, 1962, case 5), whereas in this patient it was 18 days and there was no relapse when the drug was stopped abruptly. There is no previous report of the use of dapsone in follicular mucinosis and in the absence of a satisfactory treatment a trial of dapsone is recommended in this disease when not associated with a reticulosis.

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Dermatitis herpetiformis

A CASE PROVOKED BY IODINE

ELLIS FROM AND KRISTIAN THOMSEN

Department of Dermatology, the Finsen Institute, Copenhagen, Denmark

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SUMMARY

A 68-year-old man with a 2-month itching, symmetrical, polymorphous, bullous eruption showing histological and immuno-histological changes compatible with dermatitis herpetiformis is described. The eruption could be provoked by iodine and subsided on an iodine-free diet.

The aetiology of dermatitis herpetiformis (DH) is still unknown. In an unknown way sulphapyridine or dapsone are able to control this condition without any apparent antibacterial action. It has been suggested that nearly 100% of patients with DH have an enteropathy similar to that found in adult coeliac disease. A gluten-free diet often improves this enteropathy and sometimes also the eruption, so that dapsone may be reduced or discontinued (Fry *et al.*, 1973). In a few cases hypersensitivity to milk has been observed, and in these cases the eruption has been controlled by removing milk from the diet (Pock-Steen & Niordson, 1970).

In DH the skin presents immunopathological changes which are perhaps of aetiological significance. Thus, direct immunofluorescence investigation of uninvolved skin close to lesions and of erythematous skin reveals deposits of immunoglobulin at the basement membrane zone; this is most marked at the top of the dermal papillae where the light microscope also shows the first histological changes. The fluorescence pattern is granular or fibrillar, rarely linear, and the immunoglobulin consists chiefly or exclusively of IgA. In pemphigoid the same technique reveals a linear pattern, but in this condition the immunoglobulin consists chiefly or exclusively of IgG (van der Meer, 1969; Chorzelski *et al.*, 1971). Unlike pemphigoid, DH shows no circulating antibodies against the basement membrane. However, circulating antireticulin antibodies have recently been found in DH (Seah *et al.*, 1971).

An association between thyrotoxicosis and DH has been described. Moreover, it was demonstrated recently that thyroid auto-antibodies were more commonly present in DH than in a control group (Fraser, 1970).

It is an old observation that DH is aggravated by iodine (Besnier & Doyon, 1891), and for many years iodine has been used as an empirical diagnostic test, both as an epicutaneous test and as an oral provocation test. Danlos (1898) described a 50-year-old man who developed itching after a few days' treatment with potassium iodide, and soon afterwards erythema and bullae. Even though iodine was discontinued, the eruption typical of dermatitis herpetiformis continued for 2 months. Wolter (1932) presented a case of DH in a 56-year-old man previously without skin disease following the injection of an iodine containing drug. Similarly, Warner *et al.* (1972) saw aggravation of juvenile DH caused by contrast media in four out of twelve cases. In two patients with DH, van der Meer (1969) found that dapsone was of little or no effect if the diet contained iodine.

The patient to be presented below had DH provoked by iodine, but remained without symptoms on an iodine-free diet.

CASE REPORT

A 68-year-old man who had been treated for 4 years with L-3,5,3′ triiodothyronine (Tertroxin) for presumed, unconfirmed myxoedema, was admitted with a bullous eruption. He had no previous history of skin diseases, but 2 months prior to admission he developed an itching and burning rash on both elbows. The rash subsided in a couple of weeks, but soon returned to involve also the knees and buttocks. Two weeks before admission the condition had severely deteriorated, bullae appearing after 3 days' treatment of a cold with an iodine-containing expectorant.

On admission the patient had a symmetrical eruption on the extensor surfaces of the elbows, in the axillae, on the thighs and knees as well as in the sacral region. There were erythema, papules, bullae, crusted ulcers, and in the axillae erythematous, circinate areas with grouped vesicles and small bullae. After a few days in hospital the itching and eruption began to subside and thereafter disappeared entirely. An oral provocation test was performed with potassium iodide 1·5 g daily. After 24 h the patient again developed severe itching and an eruption of grouped vesicles in erythematous areas, symmetrically on the thighs, knees, arms, and back of the neck. He was then placed on an iodine-free diet with iodine-free table salt (having previously eaten plenty of fish), and in 2 weeks the rash again disappeared. Otherwise the patient received no medication.

There were no clinical signs of enteropathy. Fat excretion and xylose tolerance were normal, and a small intestinal biopsy showed a normal jejunal mucosa. Haematology was normal, ESR 19 mm/h. Serum-thyroxine and tri-iodine–thyronine in serum were normal. Thyroglobulin antibodies or cytoplasmatic thyroid antibodies were not found.

Biopsy of the lesions on admission showed subcorneal bulla formation with acantholysis. After iodine provocation, biopsy of the new lesions revealed small sub-epidermal pustules at the tips of the papillae, containing numerous neutrophil and several eosinophil polymorphonuclear leukocytes (Fig. 1).

A closed patch test with potassium iodide 50% in white petrolatum showed at the end of 48 h erythema and oedema which persisted for another 2 days.

Immunofluorescence studies

The immunofluorescence investigation was carried out by a previously described method (Heydenreich, From & Diederichsen, 1972). Specimens of uninvolved skin close to lesions were obtained by 4 mm punch biopsy, quick-frozen and stored at a temperature of −20°C. Sections of 5μ were cut in a cryostat at −20°C and examined on the same day. Skin from a normal person, blood group O, prepared as above, was used for the indirect immunofluorescence study. Commercially prepared fluorescein conjugated rabbit antihuman IgG and IgA (Dakopatts, Copenhagen, Denmark) were used in both the direct and indirect studies. The molar F/P ratio was approx. 2·3 as calculated from the extinction ratio E 495/E 278 nm which was 0·63 with 0·30 as the lowest ratio and 0·95 as the highest. Antibody titre was 100 (i.e. 1 ml antibody absorbs 100 μg antigen). The working dilution was 1:10. The readings were made with a Zeiss microscope with a HB 200 W mercury lamp. FITC interference filter was used as primary filter and K 520 as secondary filter.

Results. The direct immunofluoresence study revealed deposits of IgA in a granular pattern at the

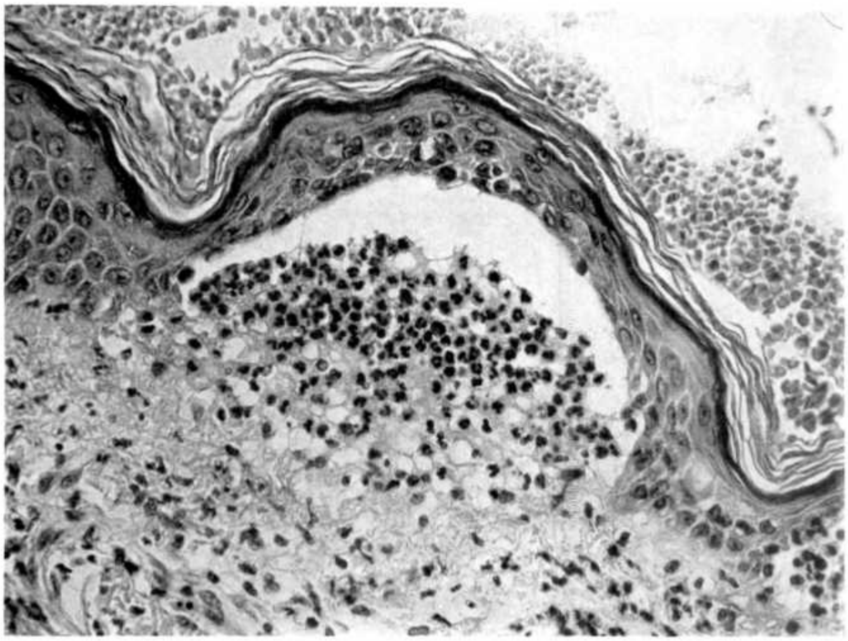

FIGURE 1. Iodine provoked skin lesion with pustules at the tips of papillae containing neutrophil and eosinophil polymorphonuclear leukocytes.

basement membrane zone (Fig. 2). IgG was not observed. The indirect immunofluorescence study did not reveal circulating antibodies against the basement membrane zone.

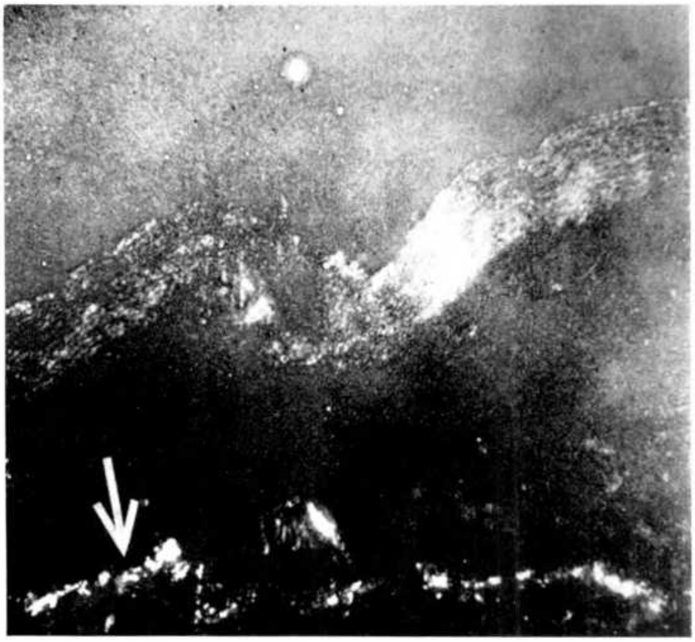

FIGURE 2. IgA-deposits in a granular pattern at the basement membrane zone (arrow).

DISCUSSION

This patient had an acute, severely itching, polymorphous, bullous, symmetrical skin disease presenting itself clinically as dermatitis herpetiformis. It was aggravated by oral iodine and subsided following iodine withdrawal from the diet. Histological examination on admission was not characteristic, showing subcorneal bullae with acantholysis, although Degos & Civatte (1961) have described such changes in DH. However, after iodine provocation the histological findings became typical of DH. Finally, direct immunofluorescence microscopy of uninvolved skin close to lesions showed granular deposits of IgA at the basement membrane zone, a finding characteristic of dermatitis herpetiformis (van der Meer, 1969; Chorzelski *et al.*, 1971).

Iodine-provoked dermatitis is well-known. It may occur as acneiform eruptions, as vegetating lesions, so-called iododerma, and as eczematous or bullous dermatitis. In cases of doubt, an epicutaneous iodine test may afford guidance, histological examination showing an eczematous reaction with spongiosis in iodine dermatitis, whereas the positive epicutaneous iodine test in DH reveals the changes of DH (Schnyder, Taugner & Rossbach, 1969). To our knowledge IgA deposition in the basement membrane zone has not been reported in iodine dermatitis.

Thus, in some cases iodine appears to be able to cause or elicit dermatitis herpetiformis, possibly in predisposed individuals. The mechanism of this action is unknown. Normally, iodine inhibits hyaluronidase, but the inhibition is reduced with decreasing iodine concentration, and in certain low concentrations iodine is even assumed to accelerate the spreading effect of hyaluronidase (Alexander, 1965); perhaps this is the very effect which occurs in the dermal papillae.

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Dermatology in the European Economic Community*

The development of dermatology in the Netherlands

J.R.PRAKKEN†

Formerly head of the Department of Dermatology, University of Amsterdam, The Netherlands

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Dermatology as a well-defined branch of medical science and practice developed rather late in Holland. In the second half of the nineteenth century, when on the foundations laid by pioneers like Willan, Bateman, Alibert, Biett, Rayer and others, dermatology came into being, the Netherlands hardly contributed to this development. This is not astonishing as doctors willing to devote their full energy to the study of skin diseases were rare everywhere; in a small country important work in this field could hardly be expected. The lack of original contributions did not imply that the medical profession ignored the development of dermatology; in Dutch medical periodicals much important work done in other countries was reviewed and discussed, sometimes at great length. At the end of the nineteenth century, dermatology followed the general trend to specialization in the medical profession. In 1896 there were already eighteen doctors in the Netherlands who confined their practice to skin and venereal diseases. On the initiative of Selhorst they founded in that year the Netherlands Association of Dermatologists (Nederlandse vereniging van dermatologen). Many of these pioneers had started in general practice or as surgeons in the navy or the army. A few had received a more or less adequate training abroad but most of them were autodidacts who had paid short visits to famous dermatological centres as a finishing touch.

It was not until the twenties of this century that dermatology was recognized as an important specialty in our State universities, but in the municipal Athenaeum Illustre (a university since 1877) in Amsterdam a full professorship of skin and venereal diseases was established in 1867. At that time there were in university hospitals on the continent only a few independent departments of dermatology and venereology in charge of a special professor. In Amsterdam the first professor was J. L. Chanfleury van IJsselstein (1819–1905). After taking his degree in Groningen he had studied for a considerable time in Paris, where he was a disciple of Ricord. In Brussels, where strict control of prostitution was reported (by Acton among others) to be very effective, he assisted in the compulsory medical inspection of prostitutes. After returning to the Netherlands he was in charge of the department for venereal diseases in the municipal hospital in the Hague and also of the medical examination of prostitutes. After a few years he was convinced of the futility of the medical supervision of prostitution and resigned. As a professor in Amsterdam he continued to be a venereologist more than a dermatologist.

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* Soon after the establishment of the E.E.C., representatives of the original member countries opened discussions on possible eventual agreement on mutually acceptable standards for graduation in medicine and for registration in the medical and surgical specialties. These discussions have of course continued in the enlarged community of which Britain is now a member. It is fair to say that despite infinite goodwill, many fundamental problems remain to be resolved. These problems arise less from any essential differences of opinion concerning 'standards', than from differences in tradition which influence the type of training given and also the scope of each specialty, and such problems have been encountered in most specialties. Since most dermatologists in the E.E.C. have little knowledge of training and practice in other countries, the publication of a series of articles describing the historical background and contemporary training and practice in each of the member countries seemed a useful contribution towards the ultimate achievement of reciprocity.
ARTHUR ROOK

† Requests for reprints to Professor J.R. Prakken, Minervalaan 73 III, Amsterdam-2, The Netherlands.

He published a considerable number of papers, mostly critical reviews of developments in syphilology. He was one of the founders of the *Nederlands Tijdschrift voor Geneeskunde*, still the leading general medical journal in the Netherlands, and was a member of its first board of editors. He was an advocate of the institution of specialized hospital departments and for the rights of patients with venereal diseases, but he did not contribute much to dermatology. The authorities in Amsterdam had shown a remarkably progressive outlook in founding the professorship but they did not keep this up. Chanfleury complained of lack of support by the municipal government and by faculty-members and in 1882 he resigned.

His successor, D. van Haren Noman (1854–1896), had a very different background. He started in Leiden as a biology student, published a few papers on molluscs and then transferred to medicine. After graduating, he was assistant in the pathology laboratory in Leiden, and then went to Utrecht as a lecturer in anatomy. Here he became interested in skin diseases. When he was appointed professor of dermatology and venereology in Amsterdam, he was in this field essentially an autodidact, but he was well trained in histopathology and bacteriology. He founded a laboratory in his department and was a stimulating teacher for his students and assistants. He also was an accomplished photographer; his most important work, 'Casuistique et diagnostic photographique des maladies de la peau', is a model of descriptive dermatology. The photographs, made on wet plates, which he himself had to cast, are excellent. His early death in 1896 was a loss for the university and for dermatology in Holland. The succession to Van Haren Noman did not proceed smoothly. There were fewer than twenty dermatologists in the Netherlands, most of them only poorly trained. It was seriously considered appointing an outsider; a surgeon with some experience in pathological anatomy and a promising young gynaecologist were the most serious candidates. The newly founded dermatological association protested and advocated the position of dermatology as a specialty in its own right. The dermatologists won the day and, in 1898, after the chair had been vacant for 2 years, S. Mendes da Costa (1862–1941) (Fig. 1), assistant of Van Haren Noman and head of his outpatient department, was appointed. This proved to be an excellent choice. Mendes da Costa was an outstanding clinician. For over 20 years he was the only professor of dermatology in the Netherlands, and a generation of dermatologists was trained in his department. In the dermatological association he was the scientific leader. He may be considered the father of twentieth-century dermatology in Holland. He retired in 1930.

From the beginning, the Department for Dermatology of the University Hospital in Amsterdam was a clinic for skin and venereal diseases. Indeed, Chanfleury van IJsselstein showed a marked preference for venereology, and it was only under his successors that dermatology was given full attention. At the end of the nineteenth century dermatology was also combined with urology; Mendes da Costa had been, before his appointment as professor of skin and venereal diseases, admitted as 'privaat docent' to the university, to teach dermatology and urology. In meetings of the association of dermatologists during the first years of its existence often papers were read on urological subjects, such as new developments in cystoscopy and renal excretion. In 1910 the urologists left the association. The link between dermatology and venereology has persisted in academic teaching, and also in practice. In the Netherlands, as in several other countries on the continent, a 'huidarts' (skin doctor) is a specialist not only for skin diseases, but also for the full range of venereal diseases. This is accepted as a matter of course; some 50 years ago a venereologist in Amsterdam, probably the only one in

Holland, advertised as 'huidarts, niet voor huidziekten' (dermatologist, not for skin diseases). Everyone knew what was meant and the offensive venereal diseases were not mentioned. The link with venereology has been an important feature in the development of dermatology. In more hypocritical times the image of dermatology suffered in the eyes of the public by the association with dishonourable diseases. On the other hand, by the necessity to combat venereal diseases, authorities have been induced to create better facilities for dermato-venereology.

In the first decades of this century dermatology showed a slow but steady growth. The number of dermatologists, all of them dermato-venereologists, increased considerably and more of them were well trained in Mendes da Costa's clinic in Amsterdam or abroad, where Paris, Vienna, and also Berlin, Hamburg (Unna) and Breslau (J. Jadassohn) were much in demand. In 1896 the association of dermatologists had eighteen members, in 1921 there were sixty-seven. Progress also showed in the scientific activities of the members. At first the meetings were almost entirely devoted to case reports and full discussion of demonstrated patients, but gradually reports on systematic research appeared in the programme. In the international dermatological journals a modest but increasing number of papers by Dutch authors appeared, most of them on work done in the Amsterdam University Clinic or in one of the well-known training centres abroad. With the exception of Amsterdam, the universities neglected teaching of dermatology and venereology. As a rule the already overburdened professor of surgery was charged with teaching skin and venereal diseases, of which he knew next to nothing. He used to neglect this assignment or to delegate it to an assistant, who was even less qualified. In

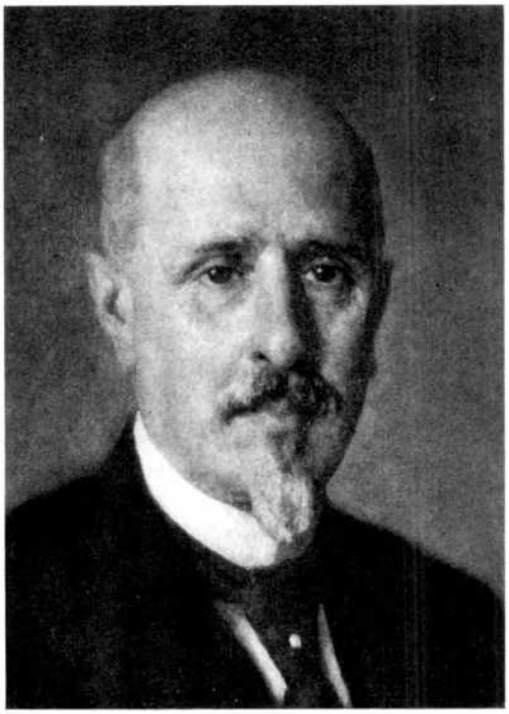

FIGURE 1. S. Mendes da Costa (1862–1941), professor of dermatology in Amsterdam from 1898 to 1930.

Groningen an exceptional situation arose, when in 1894 R. A. Reddingius was appointed professor of pathology. After his training in pathological anatomy he had worked as hospital pathologist in Amsterdam, but as a side line had practised as a dermatologist. In Groningen he volunteered to teach dermatology in addition to his normal duties; he started an outpatient department and even installed a few beds in his laboratory.

After the first world war the long overdue recognition of dermatology (and venereology) by the universities as an important branch of medicine took place relatively quickly. Full professorships were instituted in Utrecht (1919), Groningen (1924) and Leiden (1929). This not only resulted in better teaching for students, but also the founding of independent in- and outpatient departments, with their own medical and technical staff and laboratory and other facilities. By the end of the twenties, dermatology was firmly established in each of the four universities. At about this time another important development took place. On the initiative of the Netherlands Medical Association the registration of specialists became operative. Although the status of specialist had no legal status, the register made it almost impossible for a doctor not registered as such to practise as a specialist. Registration implied stringent rules for training; experience as a full-time assistant in a clinic, recognized as a training centre, was and is required. The training period varies for the different specialties; for dermato-venereology at first the minimum was 2 years; soon this was changed to 3 years, and, after the second world war, to 4 years. The University Clinics and the dermatology departments of two large general hospitals, one in Rotterdam and one in The Hague, were recognized as training centres. An examination for specialist status is not thought desirable. Even an elaborate examination is inadequate to assess a candidate's abilities, and most of us feel that mature doctors will profit more from joining in research projects than from swotting for examinations.

During the third decade of this century the conditions for the development of dermatology as a fully recognized branch of medicine were realized. The number of qualified dermatologists increased rapidly and several fully staffed and reasonably well-equipped hospital departments existed. Before the second world war, in the Netherlands East Indies, now Indonesia, a chair for dermato-venereology was established in the newly founded medical faculty of Batavia, now Djakarta. After the war there were important developments in Holland. The Roman Catholic university in Nijmegen founded a medical faculty and appointed a professor of dermatology in 1957. The Vrije Universiteit ('Free University' on protestant religious principles) in Amsterdam did the same; a full professor was appointed in 1969. In Rotterdam the clinical school attained university status in 1958 in association with the Universities of Leiden and Utrecht and in 1967 as an independent medical faculty.

It is far beyond the scope of this paper to try to review the Netherlands' contributions to dermatology since about 1930, as this would require the evaluation of well over a thousand publications. The exigencies of modern research restrict most of the important work to the University Hospitals, where manpower and laboratory and other facilities are available. In some cases very successful research has been carried out in peripheral hospitals, e.g. by M. Ruiter, who when in private practice in the provincial town of Deventer, in collaboration with the physician L. W. M. Pompen and the pathologist H. J. G. Wijers, recognized angiokeratoma corporis diffusum of Fabry as the cutaneous manifestation of a cardiovascular-renal syndrome caused by a storage disease. However, original research in the periphery is an exception; it seems justified therefore to confine this necessarily brief outline of the research done in the Netherlands to a short history of the University Clinics. The need for brevity makes it inevitable that the names of many very deserving dermatologists are omitted; only those of the heads of departments and some of their leading associates will be mentioned. Progressive circles in the present generation of students probably will consider this a deplorable relic of a paternalistic establishment, but the fact remains that a professor had, and still has, a determining influence on the research done in his department. History will show to what extent the democratization of the universities will affect the position of heads of hospital departments, and whether eventual changes will prove to be beneficial.

University of Amsterdam

In the oldest dermatological department, that of the University of Amsterdam, Mendes da Costa was succeeded in 1930 by W. L. L. Carol, who had received his dermatological training in Berlin and Hamburg during the first world war. In Amsterdam, before he was appointed professor, he was not attached to Mendes da Costa's clinic, but worked in the university laboratory of pathological anatomy, as well as carrying on a dermatological practice. He was an outstanding diagnostician and a capable histopathologist. Several rare conditions were reported by him and his co-workers for the first time in the Netherlands. He wrote a textbook to replace the obsolete books of Mendes da Costa. In 1946 he was succeeded by the author of this paper, a disciple of the Amsterdam clinic. In his inaugural speech he advocated a more functional approach in dermatology. During his professorate the department was run on the principle that members of the permanent staff should be practising dermatologists, working in the hospital on a part-time basis, thereby ensuring a close co-operation between the university and routine medical practice in the city. Another leading principle was that, in a clinical training centre, it is better to encourage the special scientific interests of members of staff than to force them into a long-term research programme. It is admitted that under this system the highest peaks in research are unlikely to be achieved, but the diversity of subjects studied has great advantages, not least for the trainees. The conviction that a psychosomatic approach is desirable in several skin conditions, led as early as 1952 to the appointment of a psychiatrist (H. Musaph) as a member of the staff. The department, though rather large (about 100 beds), was poorly housed in the old Binnen Gasthuis; in 1966 it was moved to a still older but redecorated building of the same hospital (Fig. 2);

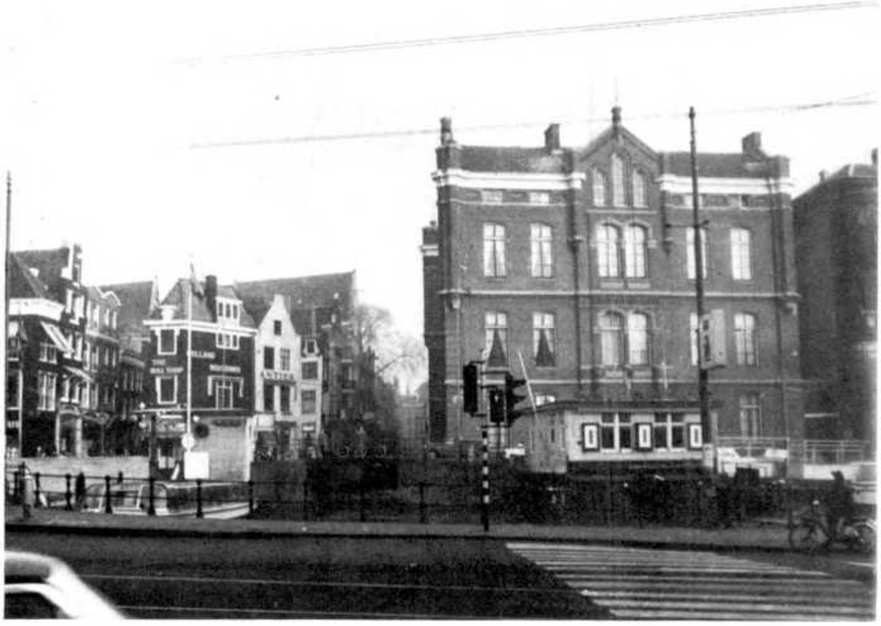

FIGURE 2. Dermatological clinic, Binnen Gasthuis, Amsterdam.

this offered sufficient accommodation. Here an innovation could be introduced: a 'night clinic' in which patients were treated, e.g. for psoriasis or atopic dermatitis, during the night, while pursuing their normal occupations during the day. Publications covered a wide range of subjects. The author of this paper continued an Amsterdam tradition by writing textbooks on venereology and on dermatology: in a second edition the subjects were combined. G. Cremer, R. Kooij, M. J. Woerdeman, J. P. Burbach, D. J. H. Vermeer, W. G. van Ketel, W. P. de Groot and H. Musaph are among the members of the staff who contributed to research over a period of many years. The author retired in 1967 and was succeeded by R. H. Cormane who was trained in Leiden as a specialist in internal diseases and as a dermatologist in Utrecht, where his work on immunofluorescence in lupus erythematosus had attacted much attention. In Amsterdam he has directed the research in his department particularly towards immunological aspects of the pathology of the skin. A sub-department for phlebology, in charge of B. J. van der Meer, meets the needs of many patients.

FIGURE 3. J. J. Zoon, professor of dermatology, Utrecht, 1945–1958.

Utrecht

In Utrecht the first professor of dermatology, Th. M. van Leeuwen, was appointed in 1918. He was a good clinician and well schooled in histopathology. He was very active in the fight against venereal diseases and for the uplift of sexual morals. His concise book on general dermatology was much used. In 1945 he was succeeded by J. J. Zoon (Fig. 3), who for several years had been a prominent figure in the clinic. Zoon was an energetic man with a domineering personality. He published widely on a great variety of dermatological and venereological subjects and promoted experimental work, e.g. by arranging elaborate installations for the study of skin temperature and metabolism in erythroderma and other conditions. Two of the leading members of his staff, J. W. H. Mali and L. H. Jansen, became professors in Netherlands universities. When Zoon died in 1958, he was succeeded by L. H. Jansen, who has published on a great number of various dermatological subjects and has promoted with great success fundamental study in dermatology. Zoon had already added a physicist and a chemist to his staff but under Jansen the work on basic problems has further developed. The head of the chemistry laboratory in his department, L. Berrens, lecturer in the biochemistry of the skin, has done, among much other work, important research on the chemistry of atopic allergens, for which he was awarded the German Robert Koch medal in 1972. E. Young is lecturer on dermatological allergy. At the celebration in 1969 of the golden jubilee of the clinic, housed during the last decade in a modern hospital building (Fig. 4), the first speaker was G. B. Dowling, who since 1957 has held an honorary degree in medicine of Utrecht university. Two of Jansen's staff members, R. H. Cormane, and E. van Dijk have been appointed professors in Netherlands universities.

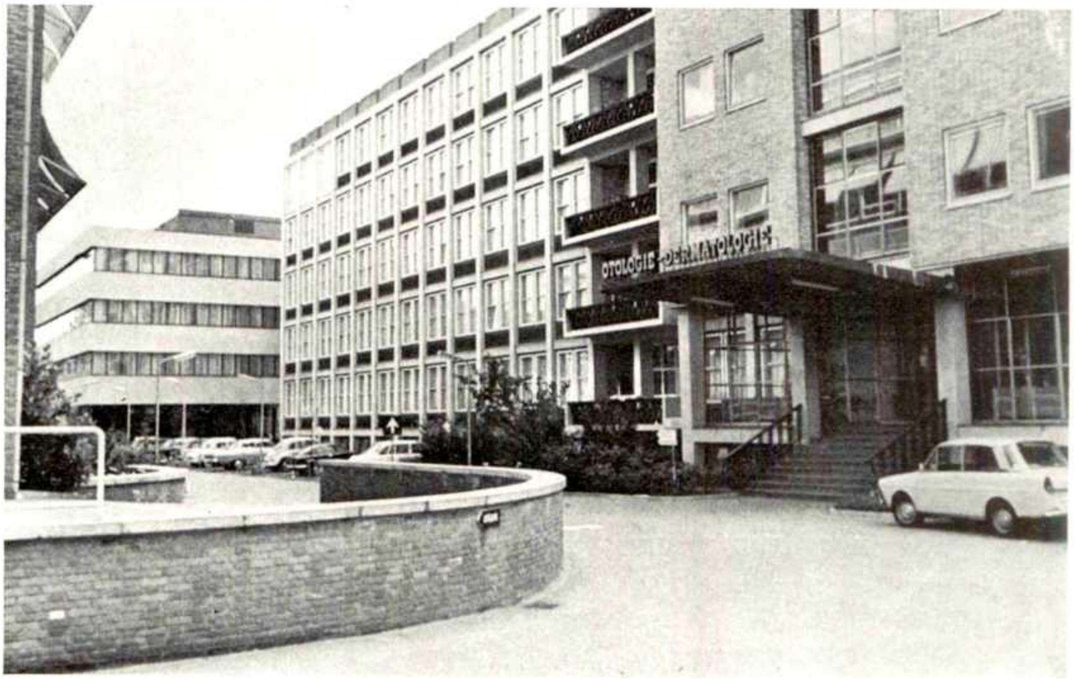

FIGURE 4. Dermatological clinic, Utrecht.

Groningen

In Groningen, the first professor of dermatology, J. W. van der Valk, a disciple of Mendes da Costa, was appointed in 1924. He had to cope with the many difficulties of installing a new department in a 'semi-permanent' building in the hospital grounds and obtaining a well-equipped laboratory. He was highly esteemed as a dermatologist and beloved by his co-workers; his early death in 1930 was much regretted. His assistant, J. A. M. Verbunt, was appointed professor in the newly founded medical faculty in Batavia. Van der Valk was succeeded by E. Zurhelle, an assistant of Erich Hoffmann in Bonn. Zurhelle was an amiable man; though not an active Nazi, during the war he showed so much affinity to the occupying forces that in 1945 he had to leave. His successor, M. Ruiter, is well known for the important work already mentioned on angiokeratoma corporis diffusum, and for concept of cutaneous allergic vasculitis and for his work (with H. M. M. Wentholt) on mycoplasmas in the urogenital tract. He also was the first to demonstrate the wart virus in epidermodysplasia verruciformis. In 1964 his clinic was moved to an impressive modern building. Ruiter retired in 1969 and was succeeded by A. H. Klokke, who had been trained in Rotterdam, and had worked for a considerable time in the tropics.

Leiden

In Leiden University a dermatology department was instituted in 1929 with H. W. Siemens, a pupil of J. Jadassohn in Breslau and of von Zumbusch in Munich, as the first professor. He was well known for his work on genetics in dermatology and especially for his studies on twins. In Leiden genetic problems held his attention, but he himself characterized his department as a 'therapeutic clinic'. Many clinical trials were carried out, especially on topical therapy in which the 'right–left method' was often used—the application of different medicaments to the two sides of the body. Siemens was a master of classical clinical diagnosis; his textbook (in German) on general dermatology was much appreciated. Siemens, who had joined in the protests of the university against Nazi activities which led to Leiden being the first of the Netherlands universities to be closed, retired in 1964. One of his co-workers, R. D. G. Pg. Simons, a prolific writer, among many other activities, edited 'Excerpta dermatologica' and a well-known handbook (in English) on tropical dermatology. Siemens was succeeded by his former pupil, M. K. Polano, head of the dermatology department in the Municipal Hospital in the Hague. He had developed that department into a centre in which several excellent dermatologists were trained and a considerable amount of research was done. He wrote, together with his father, a book on dermatology and venereology for general practitioners. His book, 'Skin therapeutics', was a much needed guide for dermatologists as well as for pharmacists. He has devoted some of his research to therapeutics and to the reaction of the skin to external influences. He was among the first dermatologists in the Netherlands to apply statistical methods to his experimental results. Under his direction the clinic in Leiden is a centre where practical dermatology and modern research are well balanced. D. Suurmond is lecturer on the permanent staff.

Nijmegen

In Nijmegen the first professor of dermatology, J. W. H. Mali, was appointed in 1957. He had been one of Zoon's prominent staff members in Utrecht, but had also worked in the physiology laboratory of the university of Groningen. He had to build up his clinic from the beginning. It is his outstanding merit that he took advantage of the possibilities offered by the preclinical laboratories in the new medical faculty. As a true disciple of the Utrecht school he did much to promote fundamental research in dermatology, as is proved by a steady flow of publications. The structure of his staff, including five chemists, two biologists, a physicist and a psychologist, is symbolic of the trend in modern dermatology. Practical medicine is also well represented; K. E. Malten, who was trained in Amsterdam, is professor of industrial dermatology, J. P. Kuiper is lecturer of phlebology. Mali has written, in collaboration with a number of his colleagues, an introduction to modern dermatology.

Amsterdam—Free University

In Amsterdam the Vrije Universiteit (Free University) appointed in 1969 as its first professor of dermatology E. van Dijk, formerly one of Jansen's co-workers in Utrecht. His department is housed in a modern university hospital. To the permanent staff belong G. L. Kalsbeek, the present editor of 'Excerpta dermatologica', and some of the former registrars of the older University of Amsterdam

(M. J. Woerdeman, W. G. van Ketel, and W. P. de Groot). In the few years of its existence the department has become a much appreciated dermatological centre.

Rotterdam

In Rotterdam, dermatologists have been active since the beginning of this century. The busy port favoured the development of large outpatient departments, offering ample opportunities for clinical training. In about 1930 this was well organized by E. H. Hermans, who became prominent in international organizations for the fight against venereal diseases. He also took the initiative in providing care for the many leprosy patients repatriated after the war. He is the author of many papers on clinical dermatology and venereology, of a textbook (with his son as co-author) and of a book on skin conditions in internal diseases, which appeared in 1973. His endeavours to introduce a classification of skin diseases are well known. In 1958, when a clinical school was instituted in Rotterdam affiliated to the universities in Leiden and Utrecht, he was appointed professor of dermatology. When this school became an independant medical faculty in 1966, Hermans was succeeded by C. H. Beek.

The Hague

In addition to the seven university clinics, one other dermatology department is recognized as a training centre, the department in the Municipal Hospital in The Hague. When Polano went to Leiden he was succeeded by R. Kooij, formerly on the staff and, after the war, temporary head of the Amsterdam clinic. He had worked for some years as a research officer in the leprosy institute in South Africa. His principal work in latter years has been on cutaneous test reactions in leprosy and sarcoidosis. Although the department is now housed in a beautiful modern hospital, research facilities are not on the level of those in university clinics.

In the Netherlands dermatology has now developed into a flourishing specialty. Some 250 dermatologists are practising and almost fifty trainees are working in the clinics. Nevertheless, it is thought in official circles that in the near future the growth of the number of dermatologists will not be sufficient to meet the increased demand for these services.

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History of Dermatology

Ramazzini and skin injury from the arum lily

DID HE PLAGIARIZE DIOSCORIDES?

J.C.MITCHELL

Division of Dermatology, The University of British Columbia, Vancouver, Canada

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'I know an apothecary in the city who, after holding a root of arum in his hand, touched his genitals; they became so acutely inflamed that gangrene resulted and a haemorrhage so acute that he nearly died' (Bernardino Ramazzini, 1633–1714).*

Perhaps this dramatic event occurred in Modena because, in 1700, the year of first publication of Ramazzini's 'Diseases of Tradesmen', the Father of Occupational Medicine moved from Modena to the Chair of Medicine at Padua.

There is relatively little concerning occupational contact dermatitis in his treatise (Wright, 1940, 1964). Bakers had strong thick hands which often became swollen and painful from kneading dough, and millers were subject to an itching dermatitis, which, with prescience, he suggested might be due to generation of mites in the flour to which they were exposed. Lime wrinkled and ulcerated the hands of bricklayers; obstruction of the pores occurred in some groups of workers. He commented also on the usefulness of bathing and the skin problems of wet nurses. And there is the chemical mutilation of the unfortunate apothecary.

The question arises as to why a pharmaceutical worker engaged in the preparation of herbal medicines was so bereft of his wits as to get the arum lily root anywhere near his genitals; there was ample warning from the text-book of Materica Medica of his day, a book which had passed through innumerable editions and versions, but which had stood the test of time all through the Decline and Fall and through the Dark Ages, as the foremost Materia Medica of the Western World: that is the text of Dioscorides (Gunther, 1968).

Physician to the armies of the Emperor Nero, Dioscorides produced his great work in the first century A.D.; the original was apparently without illustrations, but in the sixth century, illustrations were prepared by a Byzantine artist for presentation to Juliana, daughter of the Emperor of the West. The plant named Arisaron by Dioscorides was more likely *Arisarum* than *Arum* of the Araceae (the Arum family), but no matter, the plants of this family are characterized by the presence in the plant tissues of needle-like crystals of calcium oxalate. If eaten, the tubers are intensely irritating to the mucous membranes of the mouth, and it is necessary to destroy the crystals by heating them in order to prepare that edible starchy material commonly known as Arrowroot. Arum will do, and Dioscorides said of the plant: 'And collyries are made of it effectual for fistulas, but being either put in, or applied thereunto, it doth destroy the genital of any living creature'. Pliny gave the same statement, in almost the same words.

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* 'Novi ego Pharmacopoeum in hac Civitate . . .' can be thus translated (Wright, 1940).

It is not uncommon for a good teacher to illustrate a point with a story. Bernardino Ramazzini was an extremely busy man; he conducted an ample medical practice and had the responsibilities of Professor of Medicine, first at Modena and later at Padua; he was poetic, attracted by philosophy, and made original studies in geology, hydrography and barometry, and he was also a classical scholar. Despite the antagonism of his medical colleagues, his practice throve, and he found time to write the book on industrial diseases which was so successful that it was re-edited (and added to) for a hundred years.

To return to the gangrenous genitals of the unhappy druggist, did he live in Modena or Padua or amongst the pages of Ramazzini's readings in the Classics?

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British Journal of Dermatology (1974) **91**, 237.

Comment

HL-A HISTOCOMPATIBILITY ANTIGENS AND SKIN DISEASES

On page 145 of this issue there is an article associating the diseases psoriasis vulgaris and pustular psoriasis with antigens of the major human histocompatibility system, HL-A. The premise for investigating the frequency of the antigens of the HL-A system was originally based on solid evidence from studies in mice showing that the susceptibility to certain oncogenic viruses is partly controlled by the major histocompatibility system (H-2) of that species (Lilly, 1973). Since there is considerable similarity between the H-2 system of mice and the HL-A system of man, it was quite natural to expect that some HL-A antigens would occur with increased or decreased frequencies in various diseases. Furthermore, there is historical precedent for such expectations. In 1945, the distinguished British geneticist, E.B. Ford, suggested studying blood-group frequencies in human diseases because the genetic theory of natural selection assumes that no genes are likely to have only neutral effects (Fisher, 1930). Ford's prediction was proved correct by the discovery of a higher frequency of blood group O in patients with duodenal ulcer and of blood group A in patients with gastric carcinoma (Aird, Bentall & Frazer Roberts, 1953; Aird, *et al.*, 1954).

The HL-A system (major human histocompatibility system) consists of a large number of known and a still totally unknown number of closely linked loci on chromosome number 6 (Lamm *et al.*, 1974). These gene loci pass on information through RNA to the cell's surface membrane as gene products. Such gene products are most likely proteins and are easily detected as antigens on peripheral blood lymphocytes by means of humoral antibodies that have arisen during previous immunizations by blood transfusions and pregnancies. These antigens (gene products) are inherited as stable co-dominant characters controlled by two series of allelic genes on chromosome number 6. One series is called LA (or First) and the other FOUR (or Second). These names have been acquired for historical reasons not pertinent to this discussion. Since the surface antigens (gene products) are inherited as stable co-dominant characters, and since they are controlled by allelic genes within each of the two series, LA and FOUR, each individual can have only two antigens from each series. However, as there are at least fourteen different LA and twenty different FOUR antigens in the population they can be combined in many thousands of ways. The individual HL-A antigens have frequencies in the populations varying from about 50% to less than 1%.

Table 1 lists the HL-A antigens (also called HL-A determinants) with the per cent and gene frequencies of the two series, LA and FOUR.

In addition to the numbered HL-A antigens (eg. HL-A1, HL-A5) there are some numbers prefixed by the letter W (eg. W19, W5). These are current designations given by the WHO Nomenclature Committee to identify these HL-A antigens at the present time. For even more recently discovered antigens local designations are used (e.g. MK and TY). Also, it should be recalled that gene loci on chromosomes are paired, one on each of the homologous chromosomes opposite each other. The genetic HL-A information carried by one chromosome is called a haplotype and comprises one LA and one FOUR gene. One of the two haplotypes is handed on from each parent to the child usually in an unaltered form (Fig. 1). These haplotype frequencies can be determined. As one might expect, cross-overs from within one haplotype to another may occur and the frequency with which this happens is reported as the recombination frequency.

For clinical studies, the HL-A typing which is performed on freshly drawn blood uses most but rarely all of the HL-A antigens presently identified. In the study reported, all individuals were investigated for the LA series antigens HL-A1, 2, 3, 9, 10, 11, W28, and W19, and for the FOUR

TABLE 1. HL–A antigens

LA series			FOUR series		
Antigen	Frequency (%)	Gene frequency	Antigen	Frequency (%)	Gene frequency
HL–A1	31·5	0·1712	HL–A5	10·8	0·0553
HL–A2	52·3	0·3148	HL–A7	26·5	0·1400
HL–A3	27·6	0·1478	HL–A8	23·8	0·1286
HL–A9 W23	17·3	0·0917	HL–A12	25·7	0·1378
W24			HL–A13	4·2	0·0213
HL–A10 W25	3·3	0·0473	HL–A14 (Maki)	4·8	0·0244
W26	5·7				
HL–A11	10·0	0·0512			
			HL–A17 (Mapi)	8·0	0·0408
HL–A28	10·3	0·0525			
			HL–A27 (FJH)	8·1	0·0412
			W5 (R^x)	13·0	0·0722
W19 W29	18·0	0·1007	W10 (BB)	17·5	0·0926
W30	6·5	(0·0331)			
W31			W15 (LND)	16·9	0·0886
W32					
(= Li) W19·6					
'blank'	0·05	0·0228	W16–382 W16^x (U18)	5·3	0·0265
		1·0000	W18 (CM)	7 3	0·0371
			W21 (ET)	3·4	0 0173
			W22 (AA)	3·6	0·0179
			4A2^x(TT)	1·1	0·0055
			407^x	0·5	0·0023
			MK (Sabell)	1·3	0·0065
			TY	4·2	0·0213
			'blank'	0·05	0·0228
					1·0000

The antigens HL–A28, HL–A14, HL–A17, and HL–A27 have, in fact, not yet received the official HL–A prefix. However, they are likely to do so shortly, but at the present time these numbers will often be prefixed with W.

series antigens HL–A5, 7, 8, 12, 13, 14, 17, 27, W5, W10, W15, W16, W18, W21, and W22.

There are other histocompatibility gene loci being identified in man and animals that should be mentioned even though they may not be pertinent to this review at this time. Recently a third series of HL–A antigens (AJ series; see Fig. 1) have been found that, like LA and FOUR, are serologically detectable and referred to as such (SD antigens). Also, there are other HL–A gene products which can only be detected, as yet, by a special mixed leukocyte culture test. These MLC or lymphocyte dependent (LD) antigens are controlled by genes at a locus closely linked to the FOUR locus (see Fig. 1). It is worth noting that some LA, FOUR, 3rd series, and MLC genes tend to be present in the same haplotype, i.e. there is association between these four loci. It is interesting, too, that the major histocompatibility system in mice (H–2) contains a number of so-called 'immune recognition genes' (known as Ir genes). These control, in a co-dominant way, the ability of the animal to develop cellular immunity and high amounts of humoral antibodies when challenged with certain specific antigens (Benacerraf, 1974). Moreover, there is some evidence that HL–A (man), in analogy with

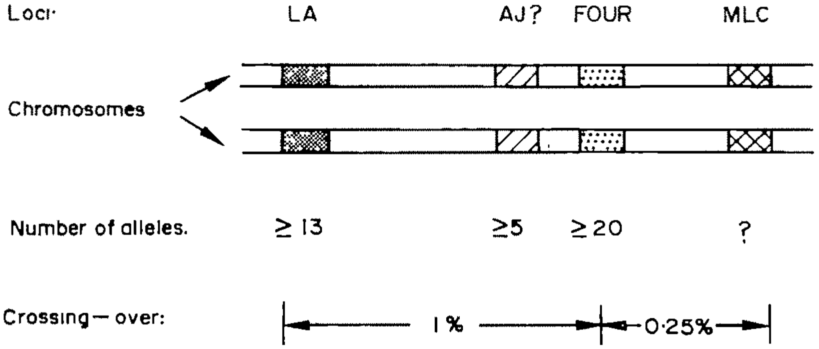

FIGURE 1. Chromosomal arrangement of HL–A genes. LA, AJ, FOUR and MLC are the loci known at the present time. In addition, there would appear to be a considerable number of immune response (Ir) genes within the HL–A complex, possibly between the FOUR and MLC loci. An HL–A haplotype is the amount of genetic information, in respect to HL–A, carried by each single of the pair of chromosomes shown.

H–2 (mice), also contains a number of immune response (Ir) genes at loci which would appear to be located in association between LA, FOUR and the MLC loci on chromosome 6 (see Fig. 1). The function of these Ir genes possibly represents the delayed hypersensitivity parallel of the variable part of immunoglobulins in humoral immunity; furthermore, they seem to influence the T and B cell cooperation in certain responses.

It is not surprising that there are associations between various HL–A antigens and a variety of diseases. The so-called 'relative risk' indicates how much more frequent the disease (eg. psoriasis vulgaris) is in individuals carrying the antigen(s) in question (e.g. HL–A13 and/or HL–A17) as compared with those lacking it. For example, in dermatitis herpetiformis the frequency of the HL–A8 antigen is increased from about 24% in normal individuals to about 50% in the patients, which gives a relative risk of 3·2 for HL–A8–positive individuals to develop dermatitis herpetiformis as compared to HL–A8–negatives. A similar increase of HL–A8 has been found in coeliac disease, and as gluten hypersensitivity seems to be involved in both of these conditions, it is tempting to speculate that an Ir gene (specific for some gluten component) closely linked to and associated with HL–A8 is involved in the development of these diseases.

However, it was more surprising to find quite strong associations between two of the FOUR antigens, HL–A13 and 17, and psoriasis. The frequencies of each of these antigens are increased about fourfold in patients with psoriasis vulgaris; also, the risk of developing psoriasis vulgaris is about five times higher in individuals possessing HL–A13 and/or 17 than in those lacking these antigens. It is most interesting that such associations were found primarily in 'early-onset' psoriasis. The HL–A13 or 17 antigens will be present in about half of all first degree relatives of psoriatics carrying HL–A13 or 17 respectively, and thus there is a higher chance that psoriasis will also express itself in these relatives. The fact that there is a normal frequency of HL–A13 and 17 antigens in pustular psoriasis also supports the view that psoriasis vulgaris and pustular psoriasis may be two distinct diseases.

Joint diseases and the arthropathies are also being sorted out by HL–A typing. Reiter's disease is definitely associated with an increase in the HL–A antigens W27. The W27 antigen is also increased in psoriatic arthritis (Zachariae et al., 1974) and has an extraordinarily high frequency (90–95%) in ankylosing spondylitis. Even reactive arthritis such as the Yersinia and gonococcal types has an increase in W27.

Lupus erythematosus, pemphigus and atopic dermatitis are being studied too, but to date the reports are conflicting and the deviations found need to be confirmed. It seems reasonable that significant correlations will arise in these and other skin diseases associated with genetic (family) predisposition.

Of the non-skin diseases, HL–A associations have also been found in chronic aggressive hepatitis, myasthenia gravis, Grave's disease, multiple sclerosis, and juvenile diabetes mellitus. Negative or doubtful findings have been found for rheumatoid arthritis, childhood asthma, and ragweed hay fever. In this latter condition, however, family studies have shown close linkage to HL–A. Contrary to expectations, a number of malignant diseases, Hodgkin's disease and leukemias in particular, have shown doubtful or negative associations—possibly because susceptibility and resistance may both be involved. (Reviews of HL–A disease associations have been given e.g. by Morris (1974) and Ryder, Staub Nielsen & Svejgaard (1974)).

Apart from the possible role of Ir determinants, two other mechanisms have been proposed to explain the associations (Bodmer, 1972). One of these is so-called 'antigenic mimicry', where a microorganism carries antigens very similar to certain HL–A antigens. As a result, individuals possessing these HL–A antigens are not able to recognize the microorganism in question as foreign, and are thus more susceptible to infection. Another possibility is that some products of certain HL–A genes may function as receptors for various viruses at the cell surface membrane. In any case, infections or abnormal immune reactions may be a common denominator for most of the above associations. These aspects should be considered when future studies of HL–A typing for other diseases are planned.

Of course, the HL–A system, which is highly informative in linkage studies for the HL–A biologist and the geneticist, plays a major role in determining the fate of allografts for the surgeon. Now the dermatologist is also being helped. Positive findings give important clues to the inheritance, subdivisions and pathogenesis of a disease and also to the possible relationships between different diseases. The clinician recognizing early onset psoriasis vulgaris can study, alert and counsel the other members of the family. Arthritis prone psoriatics may be identified before the joint disease appears and proper prophylactic measures may be developed and used. We must think again about the disease that we call pustular psoriasis. What is it, really? Our beliefs concerning the association of dermatitis herpetiformis, bowel disease and gluten hypersensitivity are firmed up a little more. We must keep alert for the significance of the immune response (Ir) gene as we deal with delayed hypersensitivity, immune complex diseases and allergic and hypersensitivity states. Now, and in the future, genetic counselling and prophylactic medicine will be additional ways by which the dermatologist will help his patients, their families and his community.

Tissue-Typing Laboratory of the Blood-Grouping Department,
University Hospital (Rigshospitalet),
Copenhagen,
Denmark
ARNE SVEJGAARD

Department of Dermatology,
University Hospital (Rigshospitalet),
Copenhagen,
Denmark
And Department of Dermatology,
University of Oregon,
U.S.A.
WALTER C.LOBITZ, JR

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News and Notices

The Queen's Birthday Honours

Dr I.B.Sneddon has been awarded the C.B.E. in the List published on 15 June 1974.

1974 Psoriasis Research Award

The Department of Dermatology, Baylor College of Medicine, announces the Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research for 1974.

This annual award of 1000 (U.S.) Dollars was established in 1957 by Carol and Henry J.N.Taub in honour of their grandparents, Mr and Mrs J.N.Taub.

Names of applicants for the award, together with supporting data, should be submitted for consideration by 1 December 1974. Results of the research must have been presented before a dermatological medical society or published in a recognized medical journal.

All communications should be addressed to:
John M.Knox, M.D.
Professor and Chairman,
Department of Dermatology,
Baylor College of Medicine,
Houston,
Texas 77025.

Investigative Group of the British Association of Dermatologists

The next meeting of the Group will be held at the Middlesex Hospital Medical School on Saturday, 25 January 1975. The meeting will be preceded by a dinner on the evening of Friday, 24 January. Members wishing to present papers should send standardized abstracts (details of which will be pre-circulated) to Dr M.W.Greaves, Department of Dermatology, Royal Victoria Infirmary, Newcastle-on-Tyne, not later than Monday, 14 October 1974.

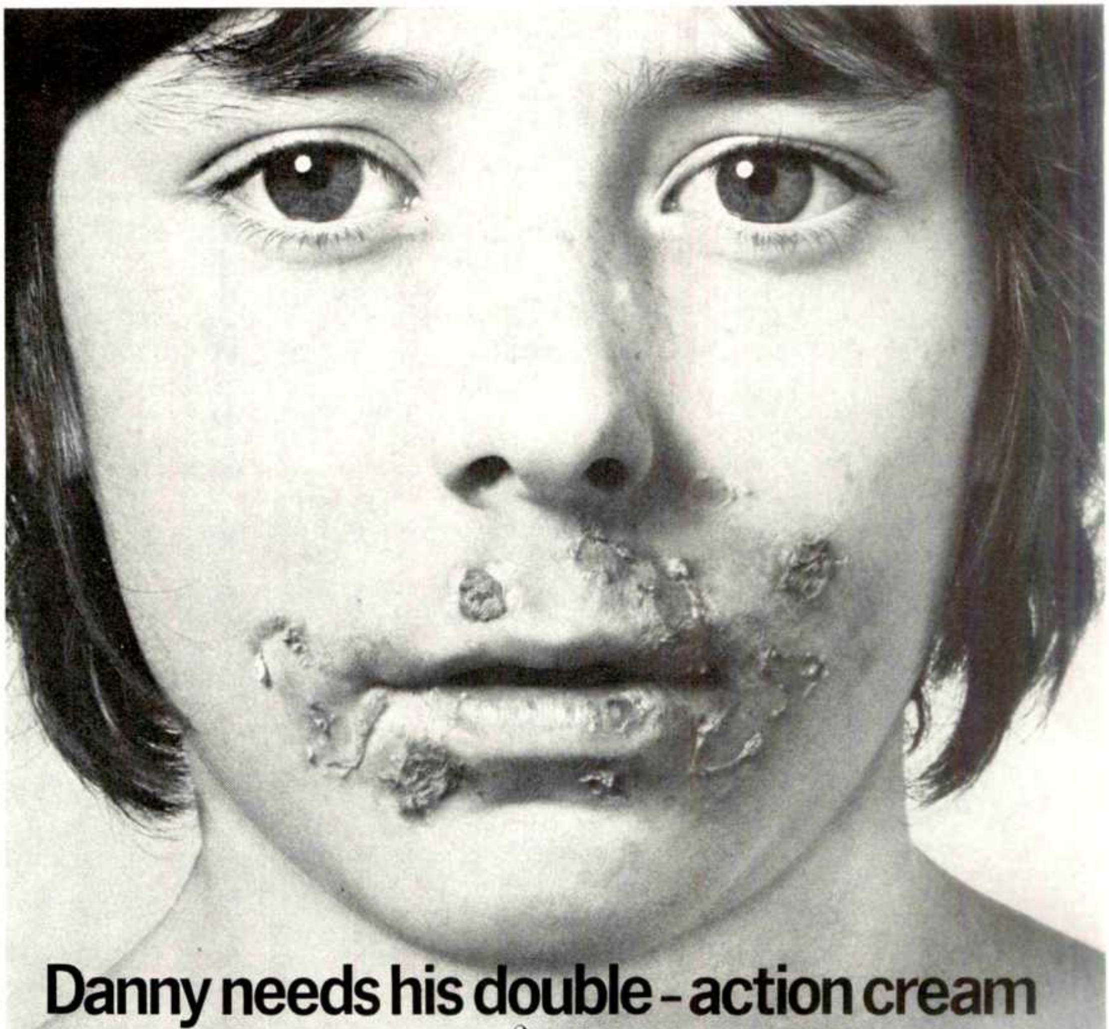
Postgraduate Medical School University of Cambridge

Annual introductory course on 'The Biology of the Skin' will be held in Churchill College from 9 to 13 December 1974. Enquiries and applications should be sent to the Secretary, Cambridge University Medical School, Hills Road, Cambridge.

APPOINTMENTS

Nottingham General and University Hospitals

Dr Roger B.Allen has been appointed Consultant Dermatologist to the Nottingham Area Health Authority (Teaching) and NOT to the Derbyshire Royal Infirmary as announced in the May, issue.



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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J. Rook, D.S. Wilkinson and F.J. Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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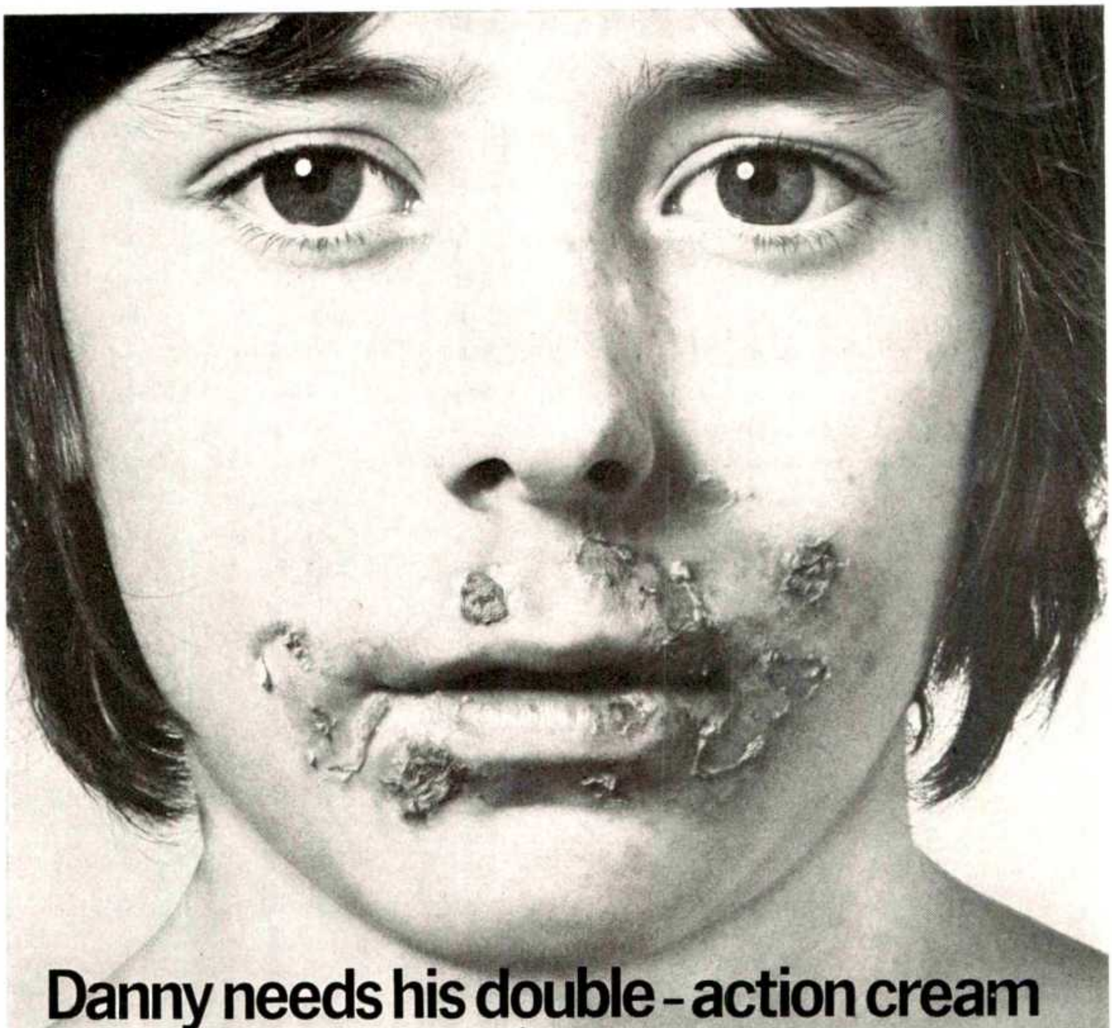

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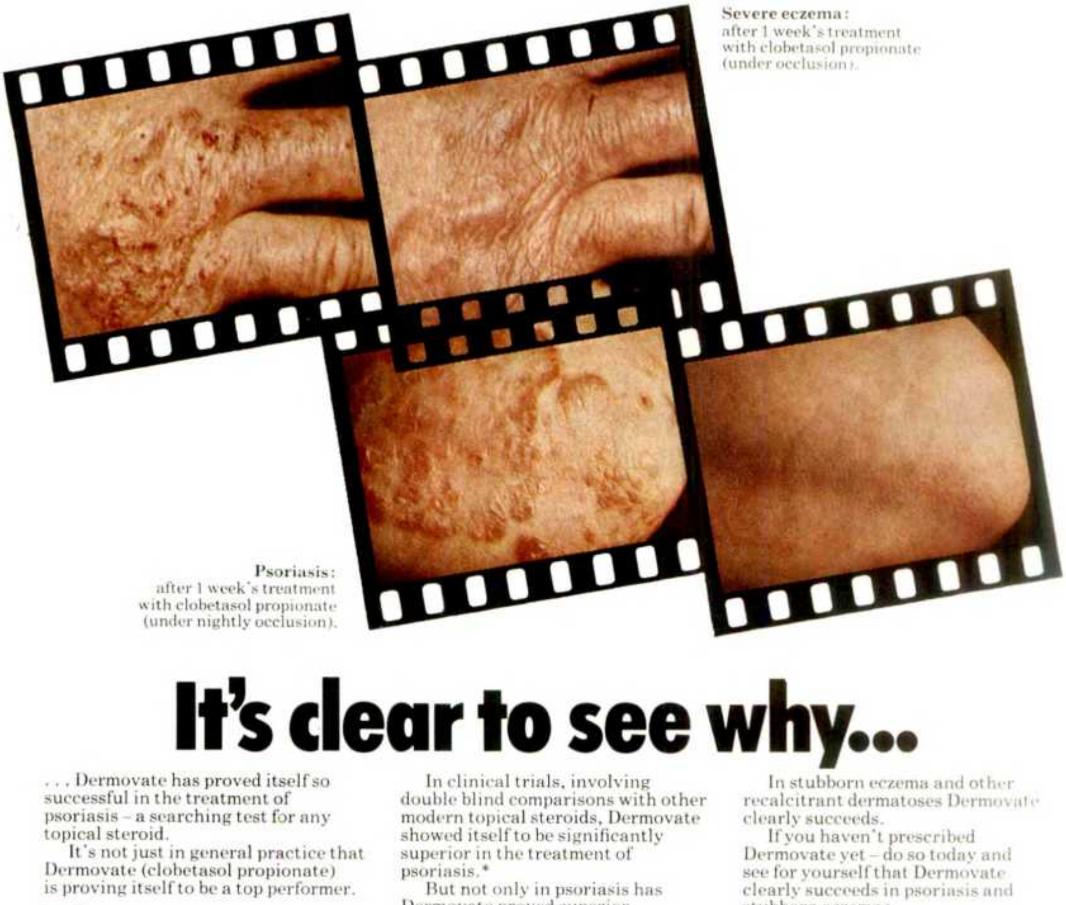

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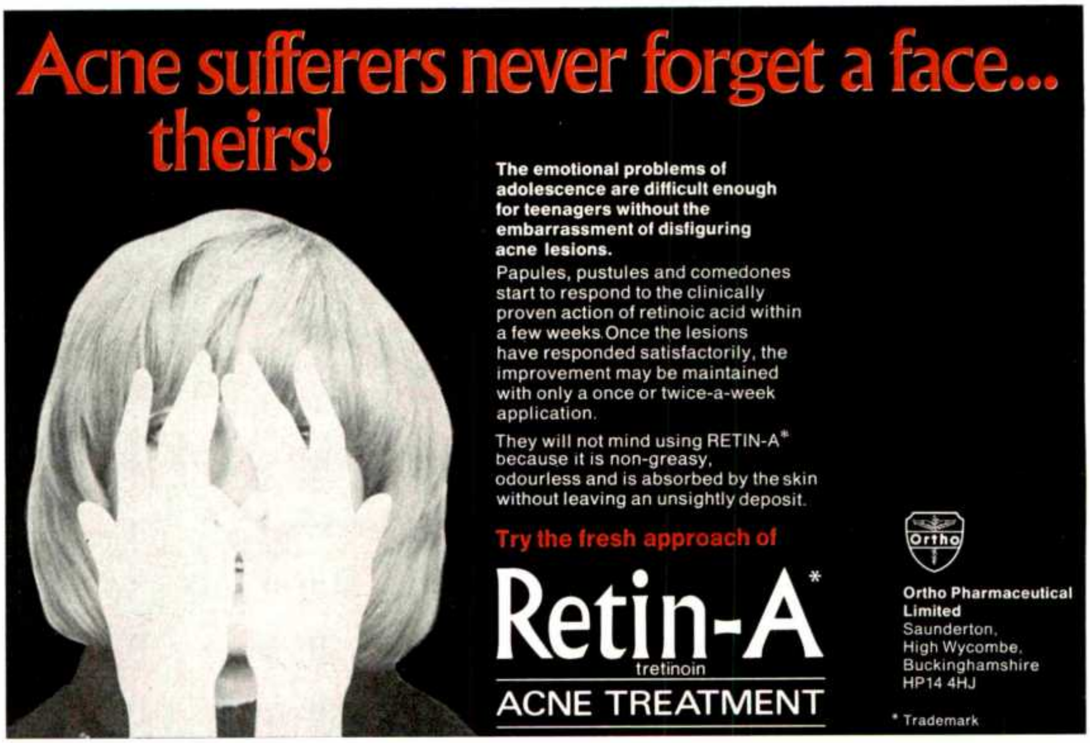

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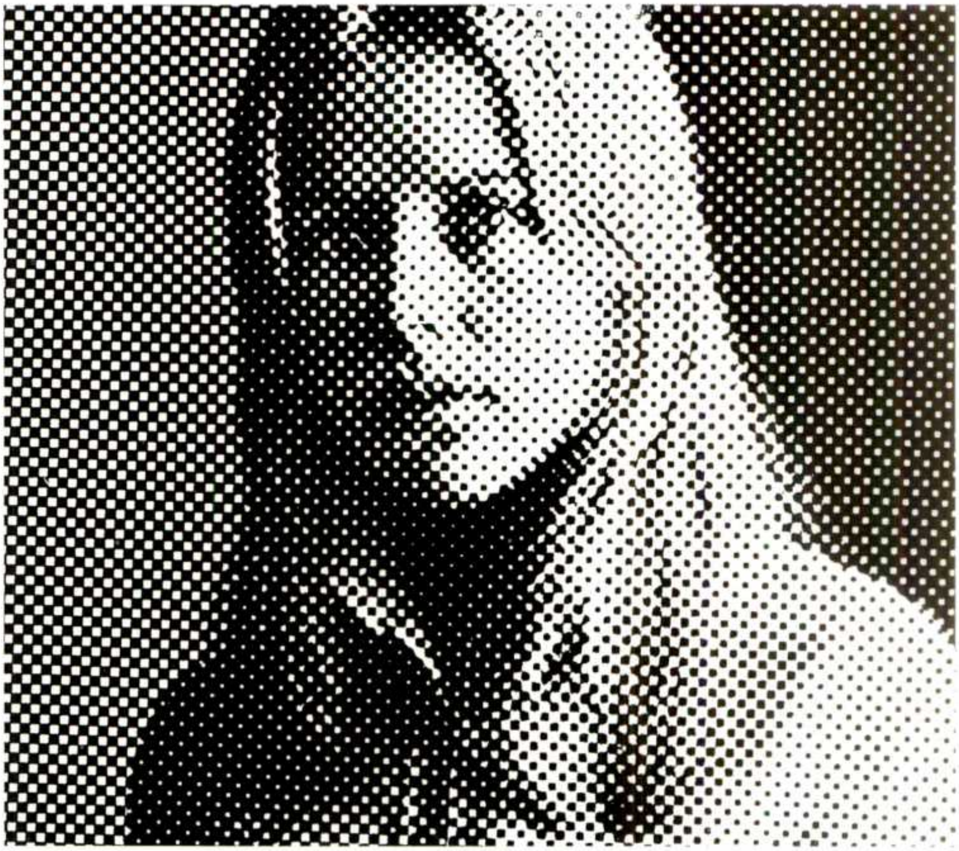

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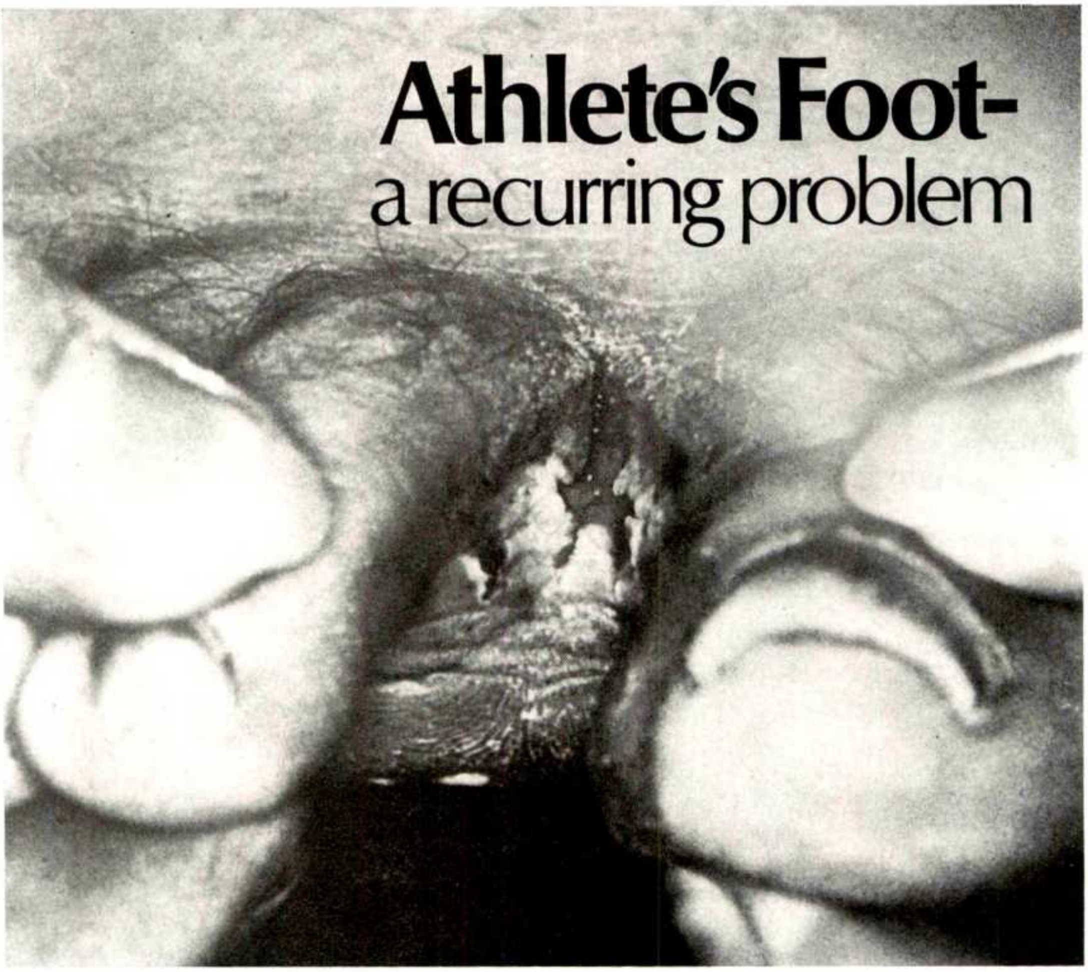

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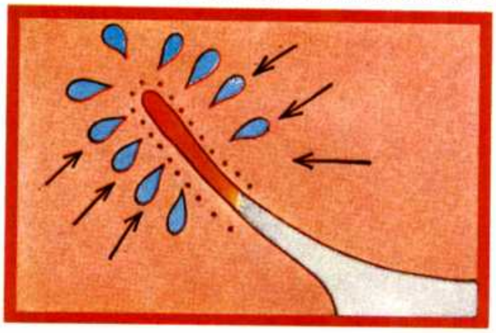

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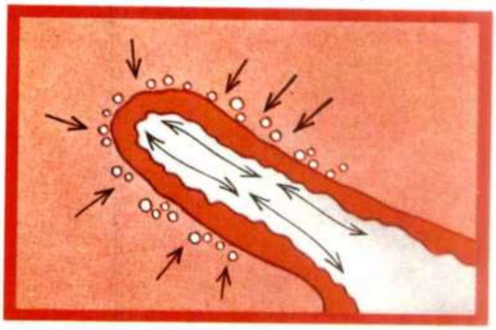

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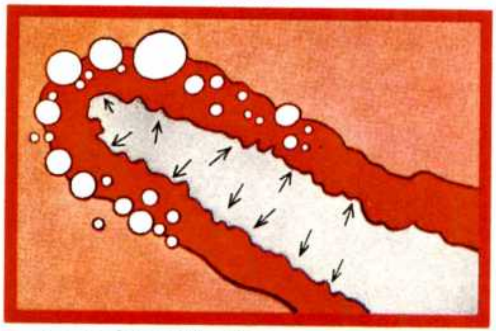
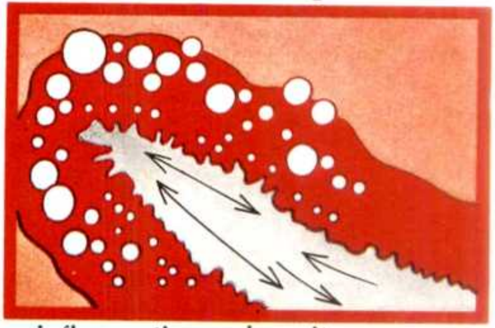


secondary infection



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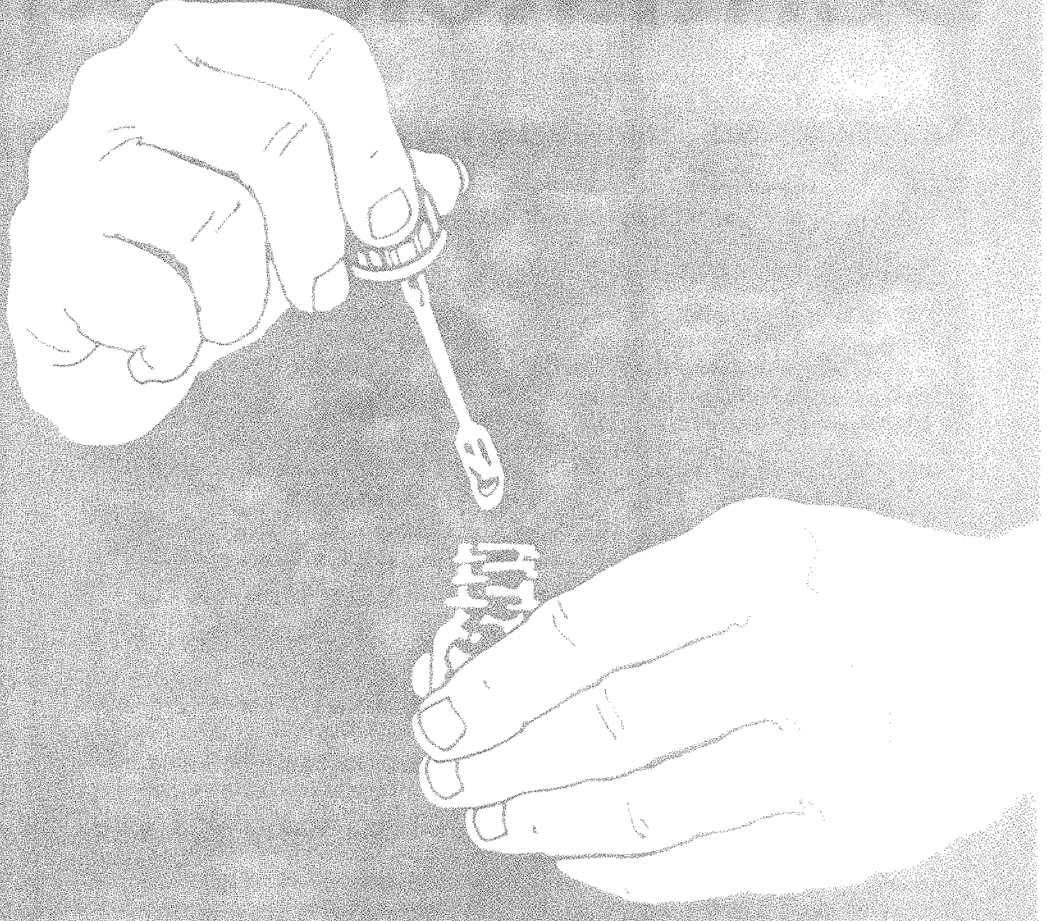

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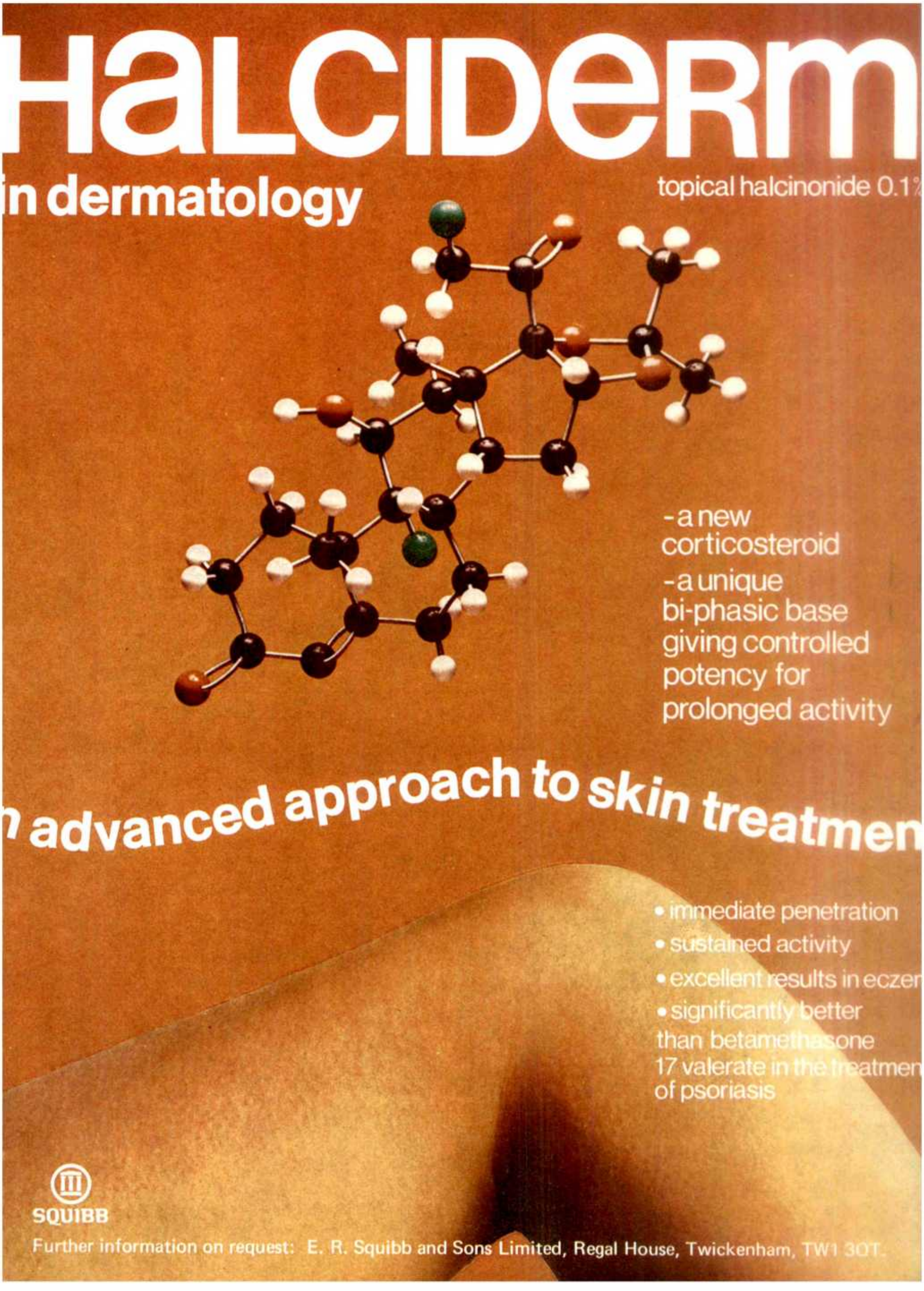

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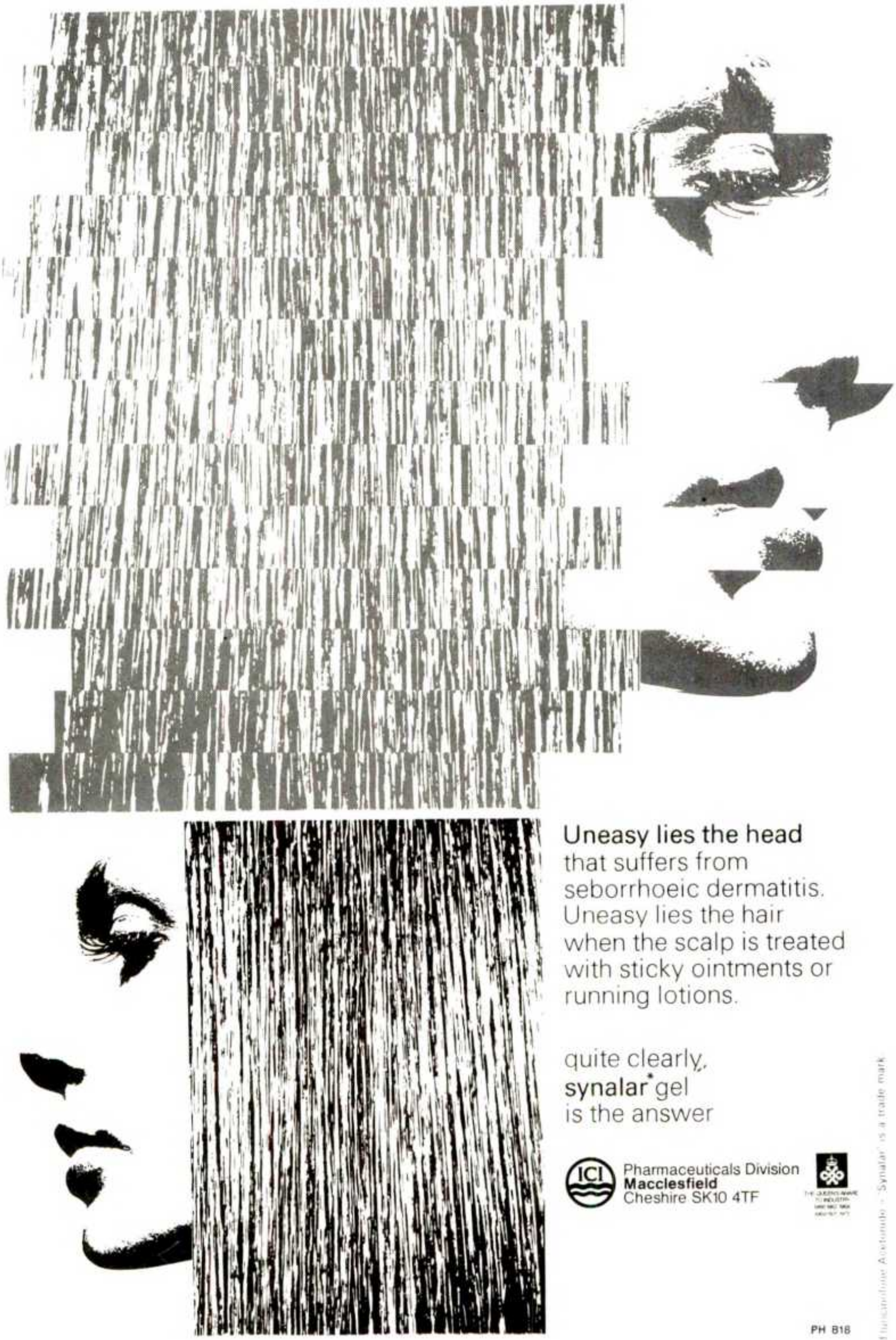

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British Journal of Dermatology (1974) **91**, 489.

Clinical and Laboratory Investigations

The Estimation of enzyme activity in living epidermal cells*

W. SCHALLA, A. ZESCH AND H. SCHAEFER

Clinic of Dermatology of the Free University Berlin, Rudolf-Virchow-Hospital, 1 Berlin-W 65

Accepted for publication 20 March 1974

SUMMARY

A method is described which permits the direct measurement of the activity of individual cytoplasmic enzymes *in vivo*. Those demonstrated include the cytoplasmic enzymes lactate dehydrogenase, malate dehydrogenase, pyruvate kinase, glutamate oxalacetate transaminase and NADP-dependent isocitrate dehydrogenase; whereas, the mitochondrial glutamate dehydrogenase and NAD-dependent isocitrate dehydrogenase were not measurable. The reactions observed in our experiments seem to be mediated by an active transport of hydrogen across the cell membrane.

In an extension of this technique to human epidermis *in situ*, we have shown an increased level of the intracellular LDH activity in psoriatic lesions, and demonstrated a marked reduction following the systemic administration of a single dose of prednisolone or methotrexate. Our method should find application in the following fields:

1. The study of regulatory mechanisms within the cell.
2. The elucidation of the cellular pathology in diseases such as psoriasis.
3. Investigation of the effects of pharmacological agents at a cellular level.

Numerous investigators have demonstrated that most of the metabolic pathways found in other organs pertain, at least qualitatively, to the skin. A number of observations, however, suggest that the overall metabolic pattern of skin is, in quantitative terms, rather different from those characterizing tissues such as liver or muscle.

The molecular biology of a cell is determined not only by the nature and the quantity of the enzymes which it contains, but also by structural parameters. Thus there is a growing need to supplement the study of homogenates (in which these structural parameters are lost) with quantitative measurements of the individual metabolic steps occurring within the intact living cell. Epidermis, by virtue of its accessibility, would seem to be an ideal tissue for such investigations.

Our team has recently developed a method which offers possibilities of this kind. Certain preliminary findings have already been published (Schaefer, 1971; 1972; Schaefer, Zesch & Nordhaus, 1972; Ottinger, Schaefer & Zesch, 1973). In this communication we wish to describe our methods, to review our preliminary results and to present certain more recent data.

---

* Parts of this paper are constituents of the thesis of M. Ottinger, H. Gauger and W. Schalla.

METHODS

(a) Investigations using isolated tissue samples. *General techniques*

Samples of liver were obtained from guinea-pigs immediately after decapitation. These were diced so as to keep the surface to weight quotient relatively constant. Specimens of clinically normal human skin were obtained at operation (mastectomy), and the epidermis separated by repeated stretching to 1·7 times its original length (Van Scott, 1952). Both liver and epidermal samples were washed three times in Ringer bicarbonate before use.

Enzyme activity was measured in the apparatus illustrated in Fig. 1. Two identical sample chambers (C_1 and C_2) were connected in parallel so that either one or the other might be included in the circuit, according to the position of the stopcocks ($TWT_{1,2}$). The concentration of reduced pyridine nucleotides in the circulating medium was monitored by means of a flow cuvette (FC), a spectrophotometer (PH), and a recorder (RC). The whole system, apart from the flow cuvette and the sample chamber in use (C_1 in Fig. 1), was cooled in an ice bath (BC). Thus the 'active volume' for freely circulating enzyme could be reduced to 3 ml, and the quotient cell bound to liberated activity increased. The tubing leading to this latter chamber was warmed by means of a heating bath (BH) kept at $32 \pm 0{\cdot}15^\circ C$.

In a typical experiment, the tissue sample (usually about 15 mg) was placed in one chamber (C_2 in Fig. 1), and the other chamber left empty as control. The reservoir (SV) was filled with Ringer bicarbonate (pH 7·4) containing additions appropriate to the particular enzyme being measured (Table 1). After a few minutes preliminary equilibration, the optical density of the circulating medium was recorded, the chambers C_1 and C_2 being interchanged each time a linear plot was obtained.

A typical curve, obtained directly from the recorder, is reproduced in Fig. 2, and a schematic drawing derived from such a curve is illustrated in Fig. 3. It is clear that the slope during periods when the 'control' chamber was in circuit (even numbered steps in Fig. 3) represents the activity of enzyme which had been liberated into the medium from the tissue, whereas the slope with the 'reaction' chamber in circuit (odd numbered steps) represents the sum of the activity of liberated enzyme plus any reaction occurring on the surface of the tissue itself. Thus the cellular activity may be calculated as the difference between these two slopes, the reaction of liberated plus cell bound enzyme minus the subsequent reaction of liberated enzyme only.

In quantitative terms, we have expressed enzyme activities as mU/mg, using the relationship:

$$\text{Activity} = \frac{10^9.V.\Delta E}{G.t.d.\varepsilon}$$

where V = volume of circulating fluid (ml)
ΔE = extinction change
G = weight of tissue sample (mg)
t = reaction time (min)
d = optical path of flow-cell (cm)
ε = molar extinction coefficient
(here $6{\cdot}2 \times 10^6$ cm^2/mol)

Individual enzyme studies

The enzymes listed in Table 1 were investigated by means of the technique described above. In general, there was no difficulty in demonstrating cellular activity relating to the cytoplasmic enzymes; both $NADH_2$-oxidising (GOT, LDH, MDH, PK) and NADP-reducing (ICDH) systems were found. Mitochondrial enzymes, however, such as GDH and NAD-dependent ICDH could not be detected with this method. In all cases, control experiments were carried out in which individual reaction partners were omitted; no enzyme activity was observed.

The mean values (±standard deviation) for the cellular activities of GOT, ICDH, LDH, MDH and PK in guinea-pig liver are shown in Fig. 4a, and the corresponding values for LDH and MDH in human epidermis are shown in Fig. 4b. As one might expect from our knowledge of the relative metabolic rates of these two tissues, we found considerably higher activity in the liver than in the epidermal samples. The mean values of the total (liberated plus cellular) activity at any time of the experiment are given in order to show the relative magnitudes, but are of no special interest as they do not reflect the continuous increase of the liberated activity itself.

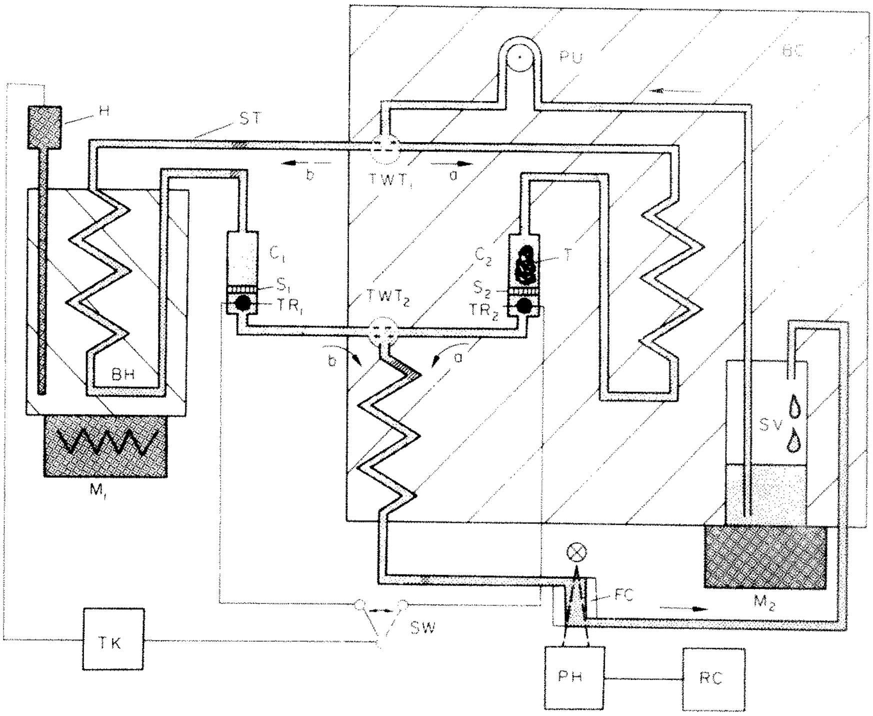

FIGURE 1. Apparatus arrangement for measurements of tissue samples *in vivo*. Direction of flow; BC, ice bath (cooled area) (6 l); BH, heating bath (250 ml); $C_{1,2}$, glass chamber (inner diameter 6 mm; length 24 mm; volume, 1 ml); FC, Helman-Micro-Flowcuvette (176–0S; volume 0·5 ml); H, heating element (soldering iron, Fa. ERSA; 25 W, 6 V); M_1, magnet stirrer with hot plate (Fa. Janke & Kunkel KG; type RCT); M_2, magnet stirrer without heating (Fa. Janke & Kunkel KG; type RCO); PH, Beckman-Spectrophotometer DB-T (λ, 340 nm) with digital indicator; PU, clamp pump (Multifix constant, type MC 1000; 220 V; 80 W; Speed of flow 10 ml/min); RC, compensating line recorder (Servogor Re 511 of Fa. Metrawatt; maximum sensitivity, 20 cm paper width=0·2 extinction units); $S_{1,2}$, sieve (mesh filter, Polyamid 1003); ST, Silicon tubing (inner diameter 1 mm); SV, storage vessel, 20 ml (glass); SW, switch; T, tissue; TK, temperature control thermostat, (Fa. Juchheim GmbH, Fulda; type Jumo Prod/1–96/bg; temperature regulation by H after adjustment of base temperature with M_1); $TR_{1,2}$, Thermoresistors (ceramics; form G 50); $TWT_{1,2}$, three way tap. Volume of system without SV, 10 ml.

Inhibitor studies

Two inhibitors have been investigated. One was ouabain (strophanthin G), which is known to inhibit membrane-bound ATPase and thus to antagonize the active transport of cations across cell membranes. The findings are illustrated in Fig. 5a; it is seen that the 'cellular' activities of LDH, MDH, and PK in guinea-pig liver were almost completely abolished by ouabain, whereas the activities of the corresponding extra-cellular enzymes were not diminished.

TABLE 1. Concentrations in the experiments

Enzyme	Test composition
GDH (Glutamate dehydrogenase; EC 1.4.1.3)	15 ml basic solution* 0·1 (0·3) ml NADH$_2$; 17 mM 0·2 ml Ammonium acetate; 7·49 M 0·5 ml α-Ketoglutarate (sodium; pH 7.5); 0·36 M
GOT (Glutamate oxalacetate Transaminase= L-aspartate: 2-oxoglutarate-ammotransferase; EC 2.6.1.1)	15 ml basic solution 0·1 ml NADH$_2$; 17 (34) mM 1·0 ml Aspartate; 7·5 mM 0·5 ml α-Ketoglutarate (sodium; pH 7.5); 0.36 M 0·01 ml MDH (25 mg/5 ml)
ICDH$_{NAD}$ (NAD-dependent isocitrate dehydrogenase; EC 1.1.1.41)	15 ml basic solution 0·2 ml NAD; 17 mM 0·2 ml Isocitrate; 80 mM 0·2 ml MnSO$_4$; 0·6 M
ICDH$_{NADP}$ (NADP-dependent isocitrate dehydrogenase; EC 1.1.1.42)	15 ml basic solution 0·2 ml NADP; 10 mM 0·2 ml Isocitrate; 80 mM 0·2 ml MnSO$_4$; 0·6 M
LDH (Lactate dehydrogenase; EC 1.1.1.27)	15 (17) ml basic solution 0·1 (0 3) ml NADH$_2$; 17 mM 0·2 (1·0) ml Pyruvate; 30 mM
MDH (Malate dehydrogenase; EC 1.1.1.37)	15 ml basic solution 0·1 ml NADH$_2$; 17 mM 1 ml Oxalacetate**
PK (Pyruvate kinase; EC 2.7.1.40)	15 ml basic solution 0·1 ml NADH$_2$; 17 mM 0·1 ml Phosphoenolpyruvate; 120 mM

* Ringer bicarbonate pH 7·4 with the following contents: 156 mM NaCl; 0·24 mM NaHCO$_3$; double distilled water up to 1000 ml; 4·02 mM KCl; 2·25 mM CaCl$_2$ 6 H$_2$O; 1·0 mM anhydrous MgCl$_2$ ** Oxalacetate solution freshly prepared (0·252 M Aspartate + 6·5 mM α-Ketoglutarate + 0·1 mg GOT in 10 ml double distilled water; preincubation 5 min at 37°C).

The other inhibitor investigated was fluoromalate, which is a competitive inhibitor of the enzyme MDH. It is seen (Fig. 5b) that the activities of both cellular and extracellular MDH were significantly reduced. These observations provided further confirmation of our view that the oxidation of NADH$_2$ which we observed occurred via specific enzyme-mediated reactions within the cell.

The Pasteur effect

This phenomenon is the inhibition of glycolysis by oxygen. It is believed to be mediated by a reduction of the available ADP and inorganic phosphate concentrations, and has only been demonstrated in preparations containing intact cells. The Pasteur effect is typical of the regulation mechanisms which enable cells to adapt their metabolism to the external milieu.

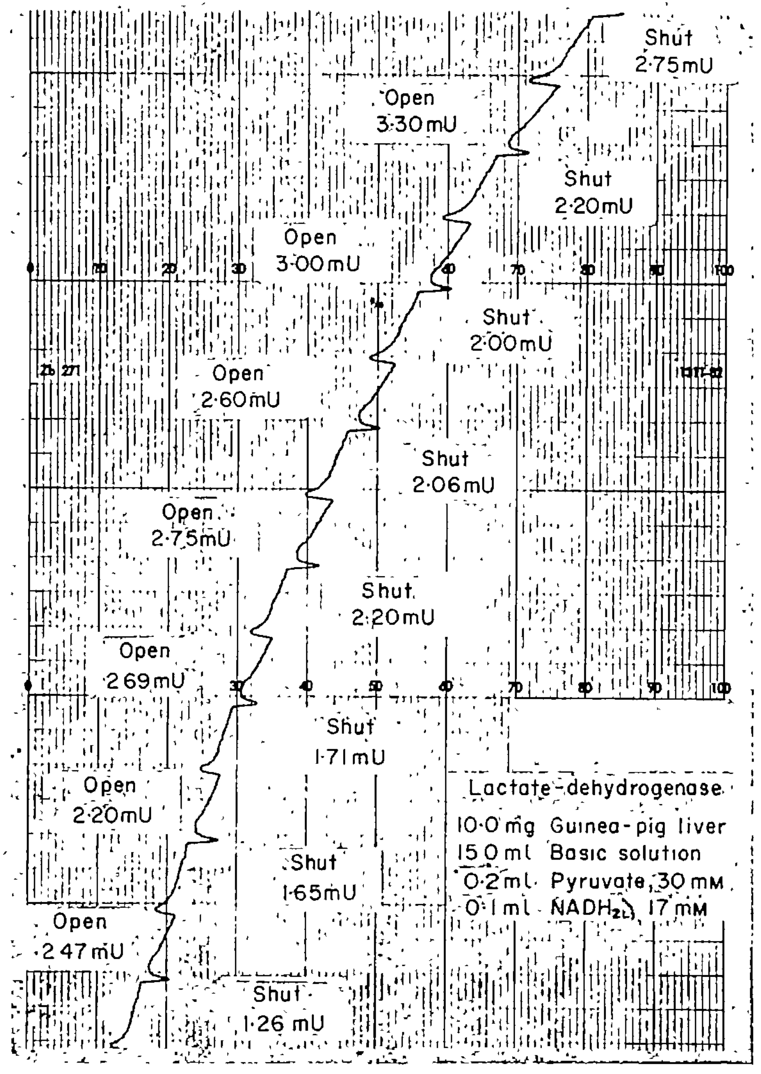

FIGURE 2. Curve obtained directly from recorder. Open, reaction chamber with tissue included in circulation; shut, reaction chamber without tissue in circulation (recording direction upwards).

We have carried out two sets of experiments. In the first, we began by displacing air from the medium by bubbling nitrogen (100 ml/min) through the contents of the storage vessel; after equilibration and measurement of LDH activity, the nitrogen was replaced by air, and LDH activity again measured.

In the second set of experiments, we began with aerobic conditions and subsequently switched to anaerobic.

A Pasteur effect was found in both cases, the activity of 'cellular' LDH being higher under anaerobic conditions than under aerobic (Fig. 6). The differences in the first set of experiments were highly significant ($P<0{\cdot}0005$), and those in the second set marginally so ($P<0{\cdot}05$). Again, therefore, we have confirmation that we are dealing with real intra-cellular events.

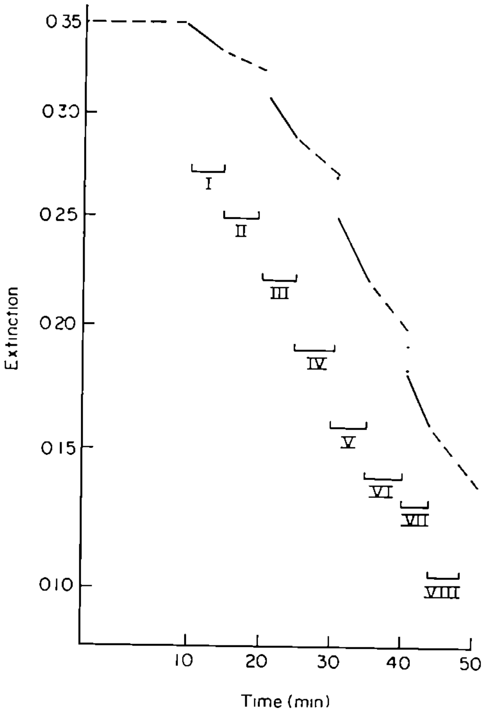

FIGURE 3. Schematic drawing of the reaction (compare with Fig. 2). Odd numbered steps, reaction in presence of tissue; Even numbered steps, liberated enzyme. Graph line recording phase: —, reaction chamber; — — —, control chamber, ... , equilibration of concentration and temperature.

NAD-penetration into the cell

At this point, we remained uncertain whether it was the hydrogen alone, or whether it was the whole pyridine nucleotide molecule, which was transported across the cell membrane.

We therefore carried out a number of experiments using ^{14}C-labelled NAD. It was found that less than 1% (about 10^{-11} mol/mg) of the NAD penetrated into the cell after 2 h, compared with a calculated transport of 10^{-7} mol/mg of hydrogen.

(b) Investigations using epidermis *in situ*

General techniques

The apparatus described above was modified for experiments *in situ* as shown in Fig. 7. In particular, the sample chambers were replaced by flat 'cups'; the heating bath was omitted since the heating capacity of the body sufficed for pre-warming.

Immediately prior to an experiment an area of volar forearm skin was stripped with 3 cm. wide cellotape (Tesafilm®) until the horny layer was completely removed (Pinkus, 1951). During the stripping process the resistance to direct current gradually fell; the end-point was determined when no further change occurred (approximately 8·7±3·5 kΩ for healthy skin, 35±5 kΩ for psoriatic skin, using a 2 cm diameter cathode on the stripped area) (Zesch, Nordhaus and Schaefer, 1972).

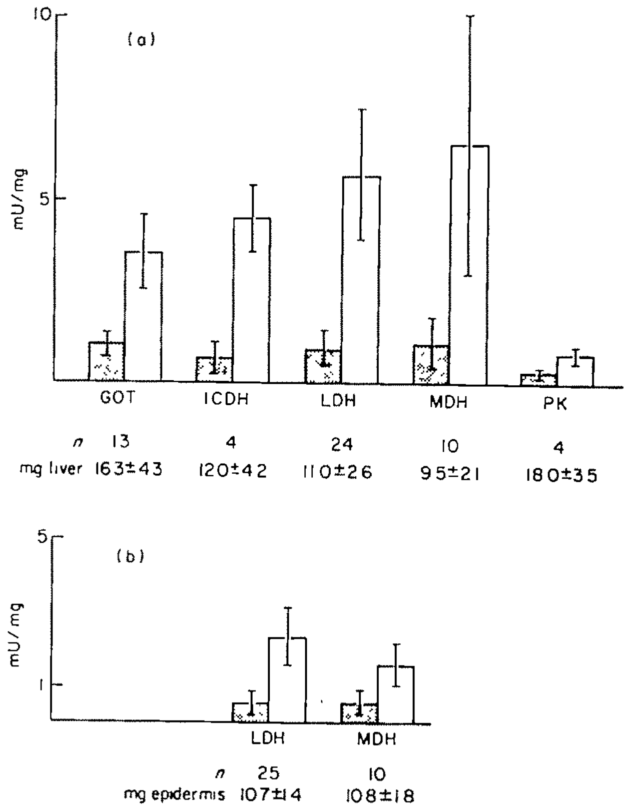

FIGURE 4. (a) Enzyme activities *in vivo* (guinea-pig liver) (mU/mg). With GDH ($n = 3$) and ICDH$_{NAD}$ ($n = 2$) no cellular or liberated activity was observed. (b) Enzyme activities *in vivo* (isolated human epidermis) (mU/mg). Hatched columns, cellular; open columns, total (cellular + liberated).

Measurements were carried out with one cup clamped onto the stripped area of skin. When disconnecting this cup, a thin fluid film must remain on the stripped skin, since otherwise drying phenomena are rapidly encountered. The control cup, although in thermal contact with an adjacent area, was isolated chemically by means of a thin sheet of plastic foil. Figs. 8 and 9 illustrate the apparatus in use.

Enzyme activities, for experiments *in situ*, were calculated as mU/cm^2 using the equation

$$\text{Activity} = \frac{10^9.V.\Delta E}{F.t.d.\varepsilon}$$

where F = surface area of epidermis (cm^2), and other symbols are as previously.

Measurements in normal and psoriatic epidermis

The above technique was used to measure the cellular activities of the enzymes LDH and MDH in the epidermis of healthy subjects and of lesions of psoriatic patients.

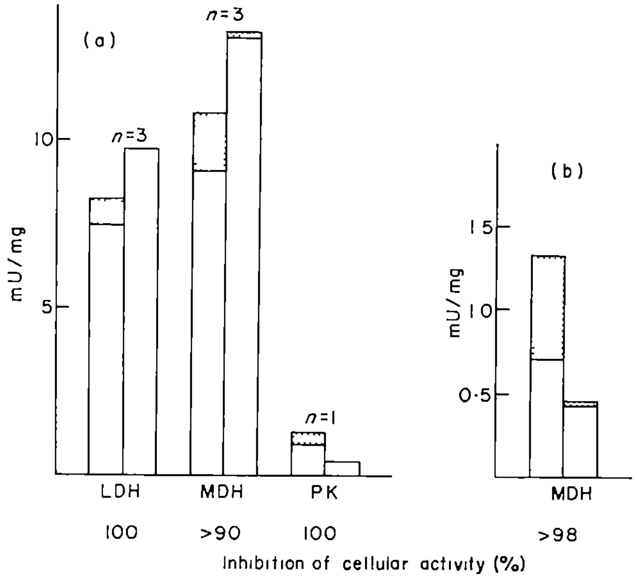

FIGURE 5. Inhibition of cellular enzyme activity *in vivo*. Each left column indicates the activities before and right column after addition of inhibitor. (a) Inhibition by 50 μg Ouabain (guinea-pig liver); (b) Inhibition by 0·1 ml, 100 mU Fluoromalate (isolated human epidermis). Hatched columns, cellular; open-columns, liberated.

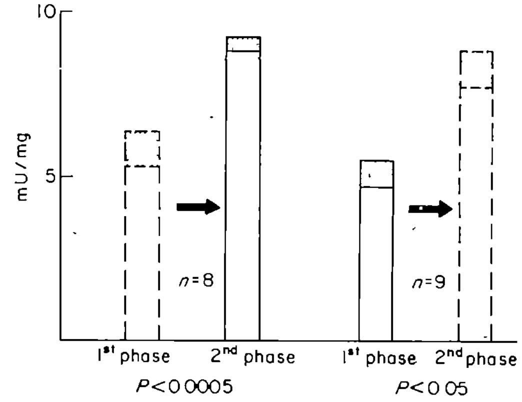

FIGURE 6. Pasteur effect with guinea-pig liver (cellular LDH-activity) *in vivo*. Hatched columns, cellular; open columns, liberated; continuous column border, ———, aerobic; broken column border, — — —, anaerobic.

The results are illustrated in Fig. 10. There was a substantial increase in LDH activity in the pathologically altered epidermis. The MDH activity was not significantly different from the control value.

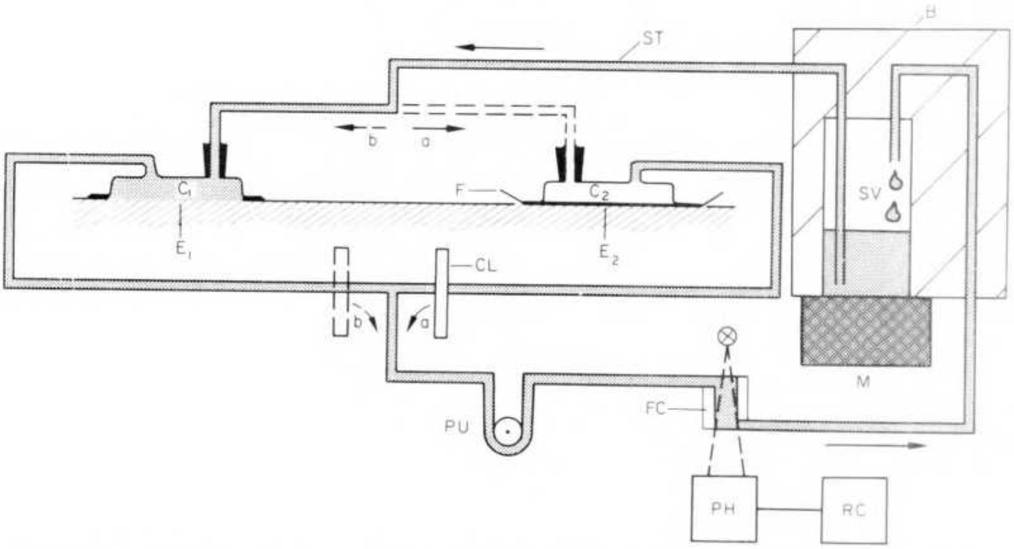

FIGURE 7. Apparatus arrangement for application on the stripped epidermis of patients *in situ*. Direction of flow a, →; reversal of flow b, →; B, ice bath (400 ml); $C_{1,2}$, flat 'cups' (specially made by the glass blower; ground glass edge: 5 mm; inner diameter 30 mm; volume 1·4 ml); CL, clamp E_1, stripped epidermis; E_2, epidermis with horny layer ($E_{1,2}$, volar forearm); F, foil (Parafilm®); FC, Helman-Micro-Flowcuvette (176–OS; volume 0·5 ml); M, magnet stirrer (Fa. Janke & Kunkel KG; type RCO); PH, Beckman-Spectrophotometer DB-T (λ, 340 nm) with digital indicator; PU, clamp pump (Multifix constant, type MC 1000; 220 V; 80 W; speed of flow 10 ml/min); RC, compensating line recorder (Servogor RE 511 of Fa. Metrawatt; maximum sensitivity, 20 cm paper width = 0·2 extinction units); ST, Silicone tubing (inner diameter 1 cm); SV, storage vessel (glass). Volume of system without SV, 6·5 ml.

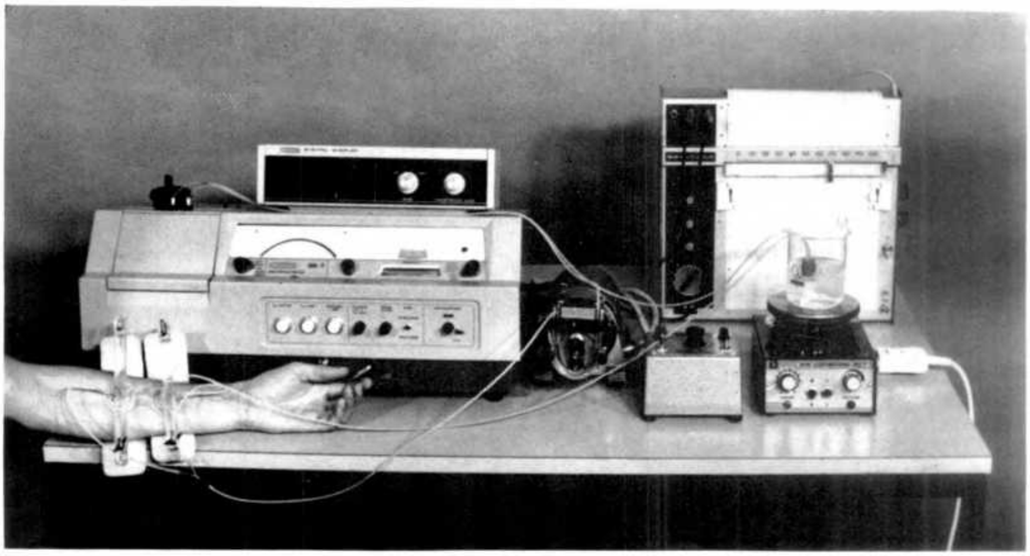

FIGURE 8. Survey of apparatus showing application to patients.

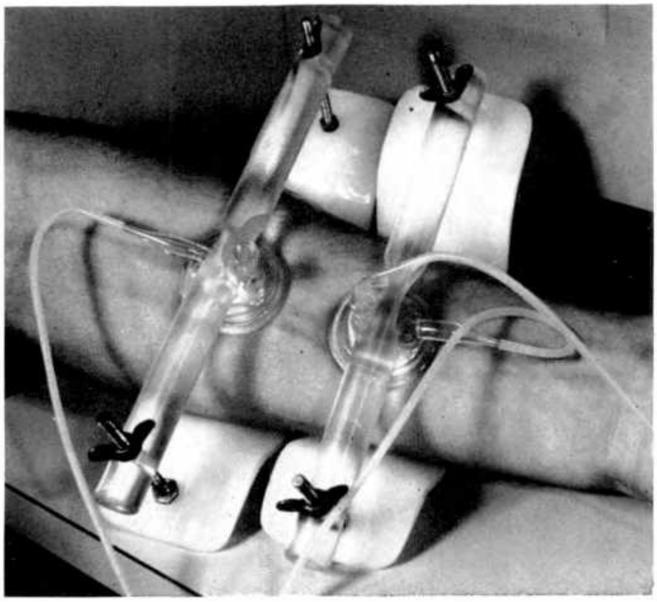

FIGURE 9. Application of method to patients: flat 'cups'.

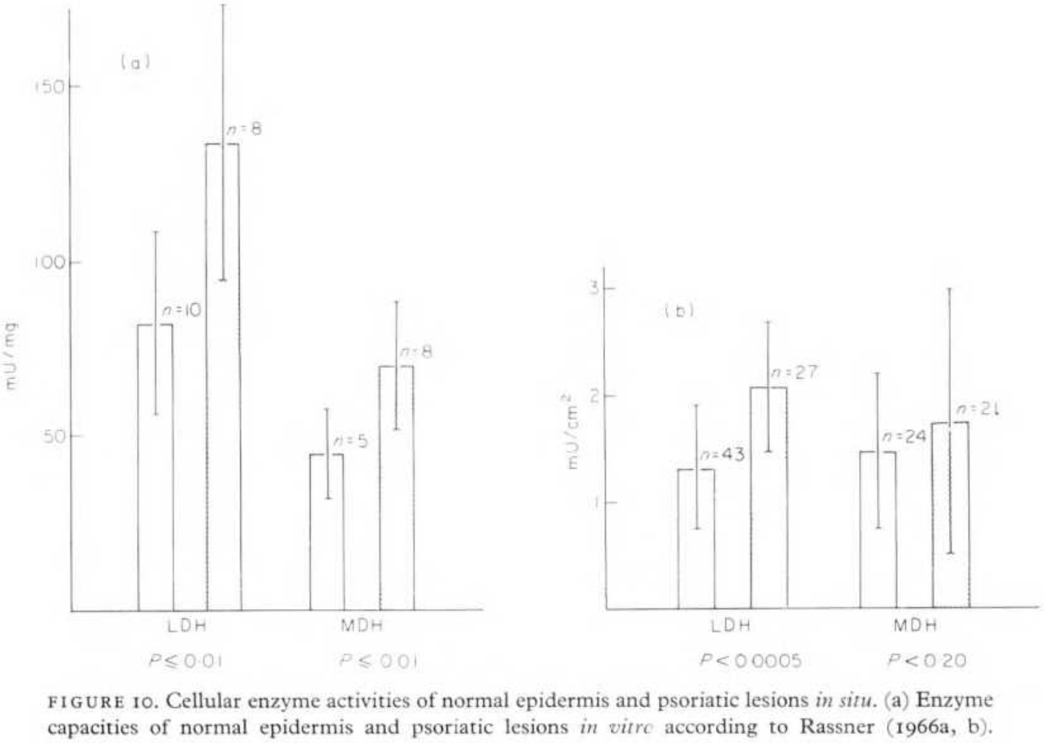

FIGURE 10. Cellular enzyme activities of normal epidermis and psoriatic lesions *in situ*. (a) Enzyme capacities of normal epidermis and psoriatic lesions *in vitro* according to Rassner (1966a, b). Stippled columns, psoriatic; open columns, normal.

Effects of methotrexate and prednisolone

A series of experiments were carried out in which the cellular activities of the enzymes LDH and MDH were determined in the epidermis before and after treatment with a single dose of either methotrexate or prednisolone.

It is seen from Fig. 11 that both of these pharmacological agents produced a rapid and drastic reduction in the cellular activities of these enzymes.

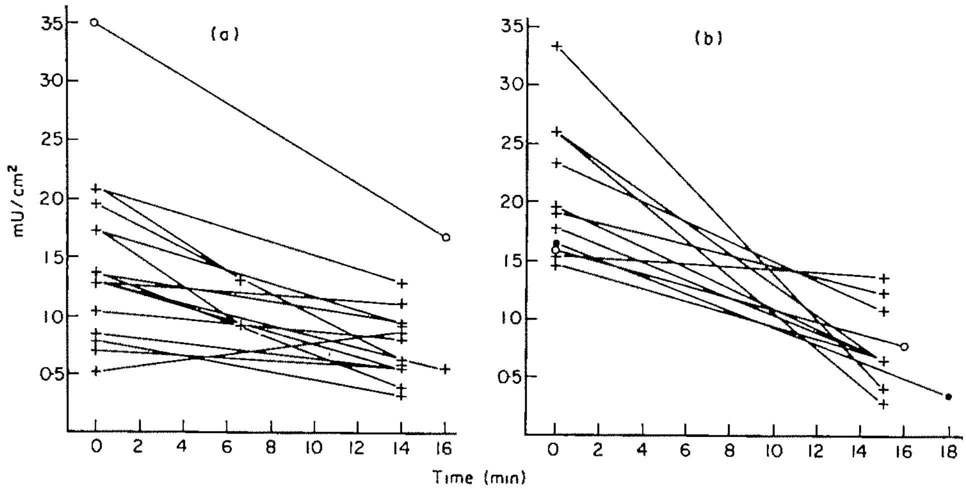

FIGURE 11. Effect of single doses of systemically administered drugs on cellular enzyme activity *in situ*. (a) 50 mg prednisolone given orally. o——o, MDH; +——+, LDH. (b) Methotrexate given intravenously. +——+, LDH 10 mg; ●——●, LDH 20 mg; oo——, MDH 10 mg.

DISCUSSION

The actual substrate turnover in the living cell is only a fraction of the theoretical enzyme capacity measured in homogenates. The cellular regulation mechanisms which are responsible for this phenomenon include the inhibition of a variable portion of the enzyme molecule and the limitations imposed by substrate transport. Regulation by variation of the rate of synthesis of the enzyme itself may also play a role, but this latter effect can be studied by the usual activity measurements on cell homogenates. The former, however, can only be elucidated by the investigation of undamaged cells.

We must first comment on the surprisingly high proportion of enzyme activity liberated into the medium. This could be caused by damage to the cell membrane and the subsequent leakage of enzyme. However, it seems to be relatively independent of both the intensity and the length of washing; in addition, the rate of efflux is fairly constant (about 0·01 mU min^{-1}. mg^{-1} for LDH). An analogous situation pertains in the experiments on epidermis *in situ*. Orfanos (1971, 1972) was unable to detect any membrane alterations in the stripped epidermis of normal skin; isolated alterations were visible in the stripped psoriatic epidermis only. Although a pathological process cannot be excluded, it seems possible that we may be witnessing an example of physiological enzyme excretion analogous to that seen in bacteria (Reisner *et al.*, 1968), suspensions of rat liver cells (Takeda *et al.*, 1964), the secretion of proline-rich protein by epidermal cells (Hay, 1964) and of hyaluronic acid and procollagen by dermal fibroblasts (Champion *et al.*, 1970).

Turning to the 'cellular' activity, there can be little remaining doubt that we are able to demonstrate and to measure specific enzyme-catalyzed reactions within the living cell. Since the reactions which we have investigated utilize pyridine nucleotides, it was clearly of importance to determine the extent to which these co-enzymes penetrate the cell membrane. The literature is conflicting on this point, some authors claiming that these molecules do penetrate cell membranes, and others concluding that the cell is impermeable to phosphoric acid esters in general. Our results support the latter view, indicating that only the activated hydrogen enters the cell. This implies the existence of an active transport system (shuttle mechanism), analogous to that of mitochondrial or erythrocyte membranes. The effects of ouabain, a specific inhibitor of cell-membrane ATPase, support this concept.

It should be noted that the transport of hydrogen to the enzyme molecule is probably not the rate-limiting step in the intact cell. This conclusion can cautiously be derived from the observations that the cellular activities of the individual enzymes are very different, whereas the overall pattern bears some resemblance to that reported in homogenates (Table 2). Other factors (as discussed above) would seem to be of much greater importance in this respect.

TABLE 2. Comparison of enzyme activities of homogenates and living cells (mU/mg)

Enzyme	Tissue	Homogenate	Intact cells This method	Cited methods
LDH	Liver	156*	0·92 (aerobic) 1·06 (anaerobic)	0·65* (anaerobic)
MDH		202*	1·09	
GOT		96·0*	1·03	
PK		15·5*	0·29	
LDH	Epidermis	82·5†	0·52	0·7‡
MDH		45·2†	0·56	

* J.R. Geigy AG (1968); (†) Rassner (1966a); (‡) Rassner (1966b).

In terms of the cell topography, it is seen that only cytoplasmic enzymes could be demonstrated. The mitochondrial enzymes GDH and NAD-linked ICDH were not measurable. Of the cytoplasmic enzymes, we were only able to observe those oxidizing $NADH_2$ or reducing NADP. Whether or not the transport of hydrogen into the cell is coupled only to NAD during influx and to NADP during efflux must be investigated further.

Our findings with regard to psoriatic epidermis are difficult to interpret at this stage. The increased cellular activity of LDH is in agreement with findings using homogenates (Rassner, 1966a, b), but the absence of any clear-cut change in MDH activity is surprising.

The investigations using prednisolone and methotrexate were carried out mainly to ascertain whether our new techniques are of value in studying the pharmacological effects of systemically administered drugs. The results are most encouraging; in particular the short time required for the inhibitory effect to become manifest contrasts sharply with the period of 8 days quoted by Halprin and Ohkawara (1967). Whether this inhibition results from a depression of enzyme synthesis or occurs via some alternative mechanism is still not clear.

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Acid phosphatase staining of the stratum corneum as a marker of damage by low irritancy compounds

T.RUTHERFORD AND A.PAWLOWSKI

Unilever Research Colworth/Welwyn Laboratory, Sharnbrook, Bedford

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SUMMARY

Biopsies of stratum corneum were taken from twenty-four human volunteers, who had been patch tested for 3 h with two experimental detergent solutions coded A and B.

The histochemical method for acid phosphatase demonstrated that both solutions damaged the stratum corneum, A more than B. It would seem that the staining reaction for acid phosphatase in the stratum corneum is a marker of the response of cells to damage by low irritancy compounds.

The irritant potential of chemicals is assessed at present by clinical observations and the use of histology or biochemistry. Clinical observation alone is not entirely reliable as it is extremely difficult to differentiate between slight changes in skin colour during inflammatory reactions. Histology and biochemistry are satisfactory for animal experimentation, but to obtain sufficient human volunteers as donors of similar biopsy material is difficult, especially for routine testing. The ability to use stratum corneum biopsy material for assessment of the irritant potential of chemicals would confer substantial advantages.

To obtain stratum corneum biopsies, Marks (1972) has used cyanoacrylate resins as biological adhesives. These are optically clear, easy to apply and cause no discomfort to the subject. The stratum corneum biopsy of normal skin allows the examination of keratinized cells in a horizontal plane. The possibility of using histochemical techniques on such biopsies as a more sensitive marker of irritant potential of chemicals has been examined. Acid phosphatase, one of the few enzymes present in relatively high amounts in the epidermis, was the enzyme of choice.

MATERIALS AND METHOD

Twenty-four human volunteers, twelve males and twelve females, were patch tested on the upper arm with two experimental detergent solutions (coded A and B) at 5% aqueous concentration for 3 h. The main difference between the solutions was the concentration of dodecyl benzene sulphonate (DOBS), A contained 21% and B 15% of DOBS; A was known to be more irritant than B in a wide range of skin irritancy tests using animals and humans. Stratum corneum biopsies were taken from the treated sites, and from an adjacent untreated site, by placing one drop of Permabond 102 Contact Adhesive on a 10 × 50 mm glass slide, placing the slide in contact with the skin for 1 min, then quickly removing it. A layer of stratum corneum cells was now adhering to the glass. The biopsies were incubated at 18°C for 30 min to demonstrate the presence of acid phosphatase using the Barka and Anderson (1962) technique.

RESULTS

Macroscopically there was no evidence of an inflammatory reaction. The microscopic findings (Table 1) show that the amount of staining produced was influenced by the treatment and that only solution A, in about half the individuals treated, caused a complete loss of the staining reaction.

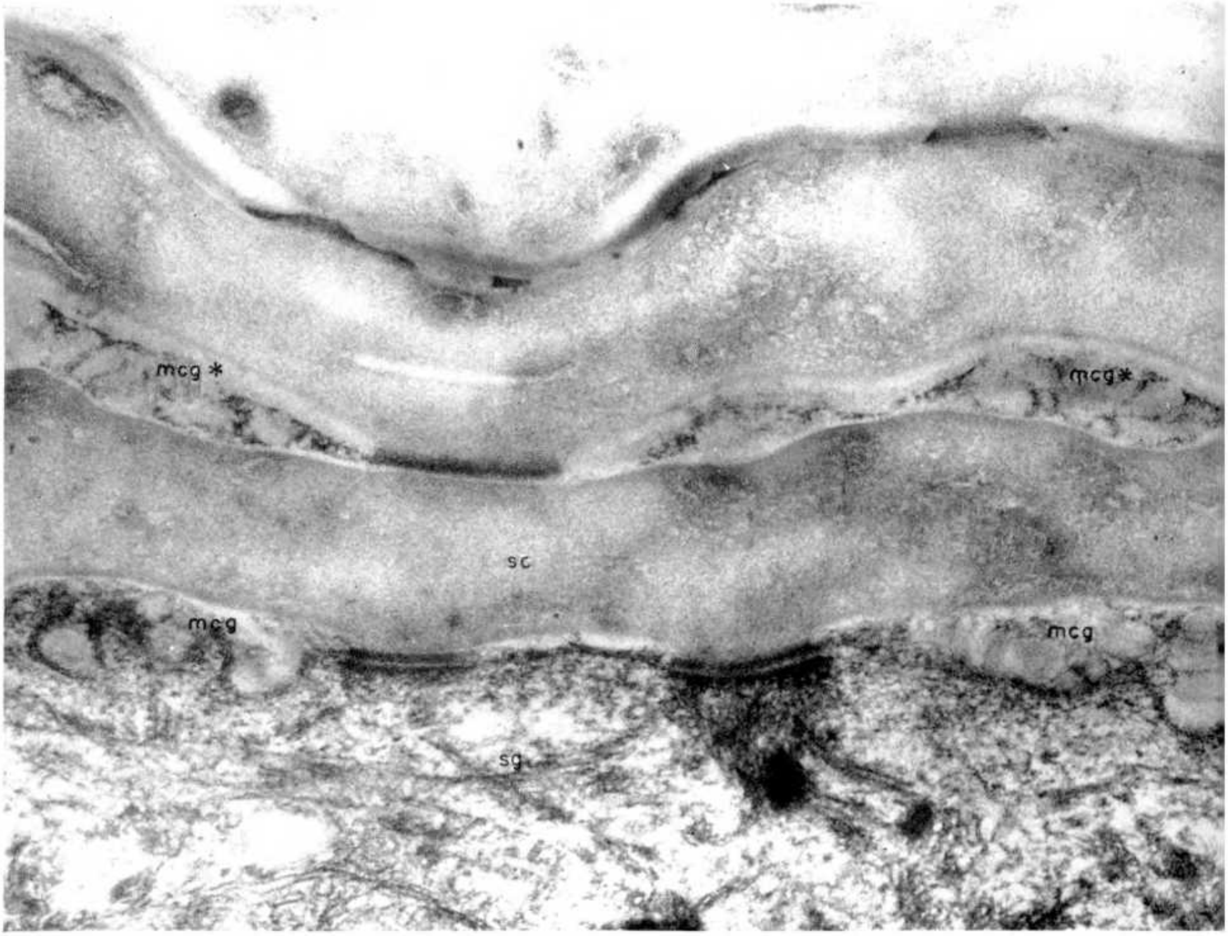

FIGURE 1. Human skin. Membrane coating granules (mcg) in the stratum granulosum (sg) and material from the membrane coating granules (mcg*) in the stratum corneum (sc) (× 72,000).

TABLE 1. Effect of a 3-hour patch test with experimental detergent solutions on the acid phosphatase staining in the stratum corneum biopsy

Treatment	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5% Solution A	++	++	−	±	±	−	−	−	−	±	−	±	−	±	−	±	−	±	−	−	±	−	−	−
5% Solution B	++	±	±	±	±	+	±	+	±	±	±	±	+	±	±	±	+	+	±	+	±	+	+	++

F, Female; M, Male; +, Control Staining; ++, Increased Staining; ±, Decreased Staining; −, No Staining.

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DISCUSSION

Although no lysosomes are present in the cytoplasm of the fully keratinized cell, membrane coating granules (mcg) are present in the lower layers of the stratum corneum (Fig. 1). It is known that mcg are synthesised mainly in the stratum granulosum and migrate to the plasma membrane (Matoltsy & Parakkall, 1965), and they have been shown to contain acid phosphatase (Wolff & Schreiner, 1970). Stereological analyses carried out in this laboratory have shown that there is an increase in the membrane coating granules when human skin is irritated by detergent solutions.

It is suggested that mild damage to the stratum corneum increases the permeability of the mcg and allows the substrate to enter the cell with a corresponding increase in staining for acid phosphatase. More severe damage alters the structural integrity of the mcg so that the acid phosphatase is slowly released from the cell accounting for the decrease in staining; very severe damage causes the complete release of acid phosphatase from the cells leaving an unstained section.

From the results presented above it is concluded that both solutions damage the stratum corneum, A more than B. This may be a contributing effect to their overall irritancy as demonstrated by other skin irritancy tests done in this laboratory using solutions A and B on animals and humans.

Thus the staining reaction for acid phosphatase in the stratum corneum would seem to be a marker of the response of cells to damage by low irritancy compounds.

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The stratum corneum—Some structural and functional correlates

FRITZ A. SCHELLANDER AND JOHN T. HEADINGTON*

Institute of Dermatology, St John's Hospital for Diseases of the Skin, London WC2H 7BJ

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SUMMARY

The human skin surface outside the areas bearing dermatoglyphics has been studied and an attempt made to explain the surface pattern in simple geometric and functional terms.

The basic geometrical unit is a rectangle or square which in turn is subdivided by one or two diagonals. The triangles and diamonds thus formed undergo varying degrees of collapse under tension. These collapse units are sometimes composed of smaller polygonal compressible sub units with a distinctly honeycomb appearance. The hexagonal shape of the individual horny cell is an ideal smallest structural unit within this geometric system. The regional differences are considerable, but can be explained by the regional differences in the mechanical requirements. These features are of considerable functional significance for the stretching and deformation of the essentially inelastic stratum corneum.

It has been known for many years that almost all of the skin surface is patterned. The configuration of the ridged skin of the volar surfaces, known as dermatoglyphics, has been studied and described in detail. By contrast, however, the fine topography of the comparatively enormous surface of the non-volar skin of the remainder of the body has received little attention. Old reports (Pinkus, 1927) and reviews (Montagna, 1962; Kligman, 1964) on the subject are limited to fragmentary morphological descriptions of different patterns in different areas, while more recent investigations (Plak & Maron, 1967; Sarkany, 1962; Marks & Dawber, 1971; Marks & Saylan, 1972; Kuokkanen, 1972) have not added substantial new findings. Most significantly, no dynamic structural–functional concepts of surface patterns have emerged from the preceding essentially descriptive studies. Moreover, old studies have been concerned with static or resting patterns; there has been no systematic examination of surface patterns deformed by vectoral forces.

While it is supposed that fascial attachments of the dermis contribute to the formation of major skin creases and that Langer's lines represent a dimension of dermal elasticity (Cox, 1941), the important dermal–epidermal interactions which determine the myriad of fine folds and creases marking the microstructure of the stratum corneum remain obscure.

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* Reprint requests to: Dr J. T. Headington, Department of Pathology, University of Michigan Medical Center, 1335 East Catherine Street, Ann Arbor, Michigan, U.S.A.

This study describes details of the fine structural patterns of the skin surface in areas other than the palms and soles. The findings support a theory that the surface pattern is fundamentally one of geometric simplicity in which regional differences can be explained by the differences in the local mechanical requirements. Deformation of the essentially inelastic though pliable horny layer is achieved by an intricate geometric folding along pre-existing lines. This establishes the skin surface pattern as a highly ordered and symmetrical geometric design of considerable functional importance.

MATERIAL AND METHODS

The sampling method used in this study was the so-called skin surface biopsy technique (Marks & Dawber, 1971). The technique consists of placing a small drop of a cyanoacrylate adhesive (Permabond, Permabond Adhesives & Sealants (U.K.), 33 Clarence Street, Staines, Middlesex, England) onto a clean glass slide. The slide is then quickly pressed onto the skin, held in place for 10–30 s and then torn away. Several layers of stratum corneum adhere to the slide with near perfect morphological preservation. Direct examination reveals the reverse or undersurface of the corneal layer. The specimen is sufficiently thin and translucent to be inspected from both aspects with no rpparent difference in the light microscopic appearance of the surface pattern.

The specimens were not stained or fixed. The slides were examined without coverslips by conventional and stereomicroscopy. Photomicrography was carried out by transmitted light using reduced illumination with a Zeiss photomicroscope.

The skin surface samples were collected from many different areas of the body from a large number of volunteers and from skin stretched in various directions under different degrees of tension. In a number of instances successive strippings were taken from the same site.

RESULTS

In almost every area of the body surface the skin discloses a geometric pattern. The pattern varies considerably in different areas and in some places the basic pattern is not immediately obvious. However, in most parts the skin markings reflect a simple geometric pattern and the local differences are likely to be due to differences in the regional mechanical requirements.

The fundamental structural change which converts the potentially featureless horny layer into one of geometric complexity is a linear fold or crease of variable magnitude. The vertical direction of the crease is downward or inward towards the dermis. The horizontal direction is not flat, but curved upwards. These two features represent a built-in redundancy which is essential for deformation of the skin surface (Fig. 5).

These linear folds cross each other giving rise to an intricate lattice-like pattern. Within limited areas the geometric patterns of creases are more or less isometric. The simplest repetitive figure is a square or a rectangle (Figs. 1, 4a and 5a). These rectangles are in turn crossed by one or two diagonals, thus subdividing each of these rectangles into two or four least triangles which represent the smallest functional geometric units (Fig. 1b). Each least triangle encompasses a variable number of horn cells. Triangles may be structured in one of two ways; either without further sub units, thus producing a uniform or featureless pattern within each triangle, or with round or polygonal sub units producing a distinctly honeycomb appearance (Figs. 2 and 4b). Each of these sub units is itself composed of a variable number of horny cells, which themselves are geometric with either hexagonal or pentagonal outlines (Fig. 6). The presence of these sub units within triangles varies according to the site of the skin specimen, but they were found more commonly in sites with pronounced flexibility (wrist, knuckle, instep, etc.) and less commonly over flat surfaces (forearm, shin). The major system of opposing and parallel lines in one plane usually represents the macroscopically visible skin creases.

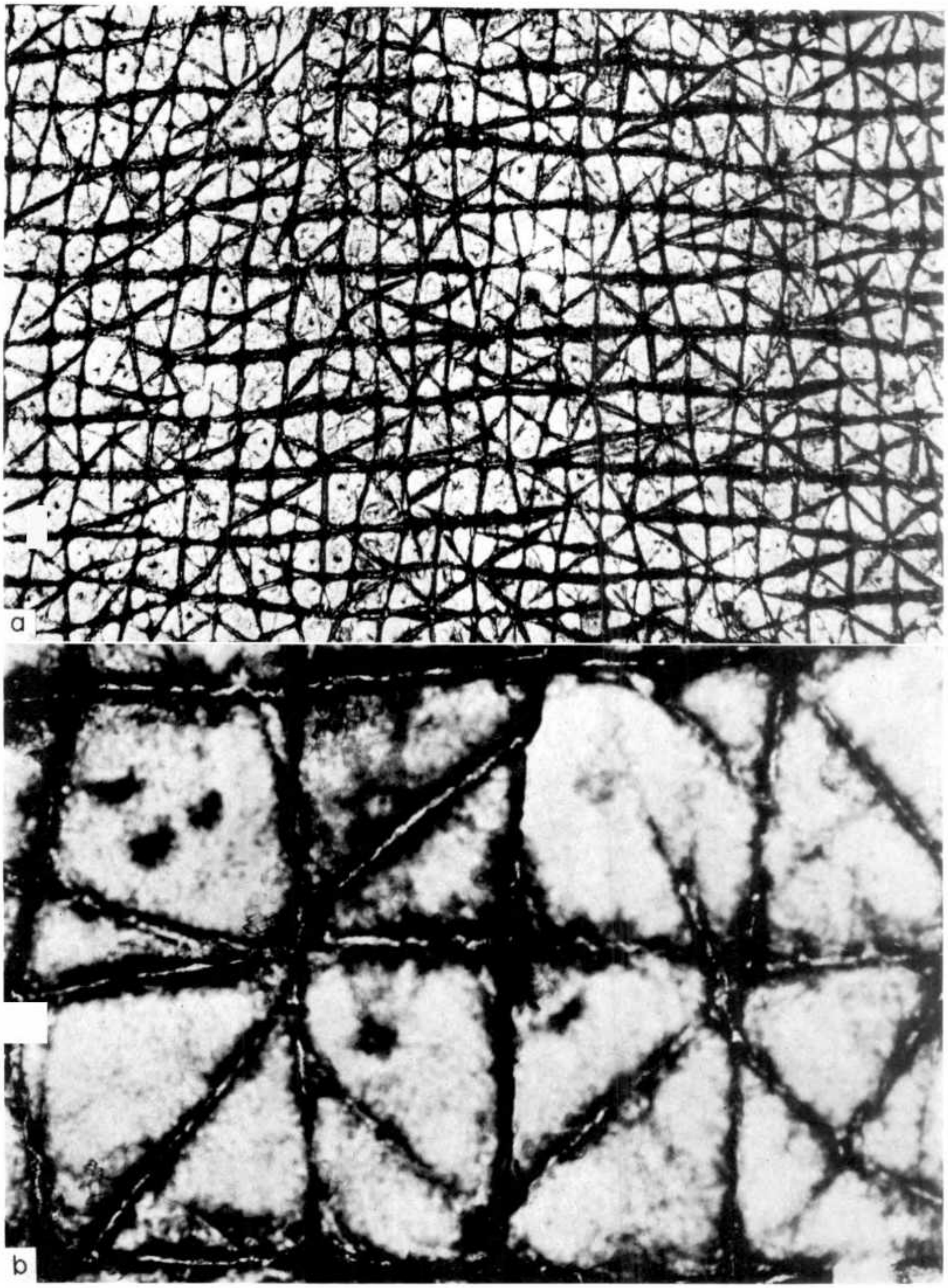

FIGURE 1. (a) Skin surface pattern (forearm) showing regular lattice structure composed of rectangles with diagonals (× 8). (b) Detail of (a) to show regularity of design. Triangles filled with evenly arranged horn cells (× 50).

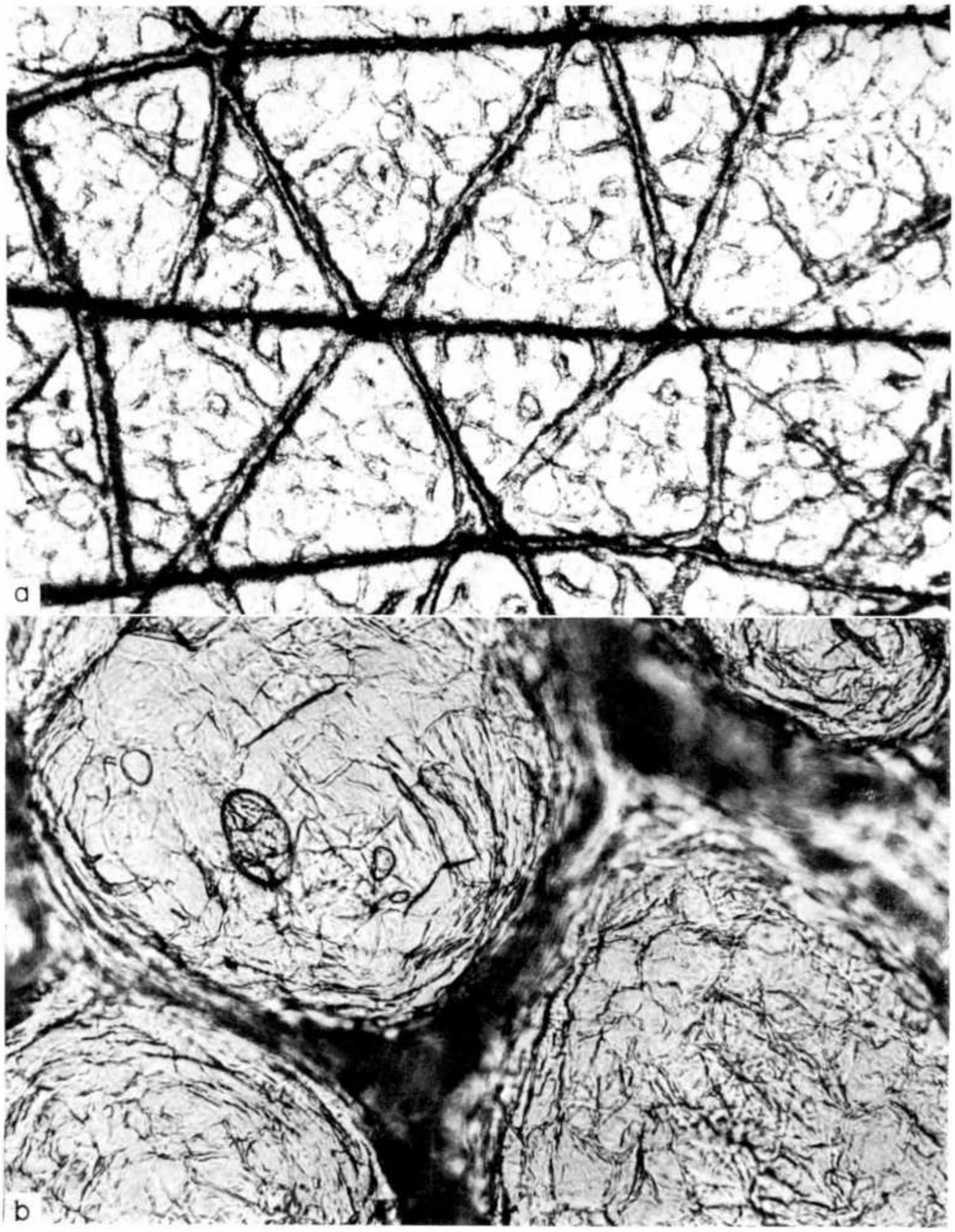

FIGURE 2. (a) Detail from instep. The parallel lines (horizontal) represent the macroscopically visible skin creases. A definite attempt is present to form equilateral triangles. Each triangle is subdivided into smaller round or polygonal (compressed) units (× 50). (b) Detail of round–oval sub units. These are the smallest deformable units (× 150).

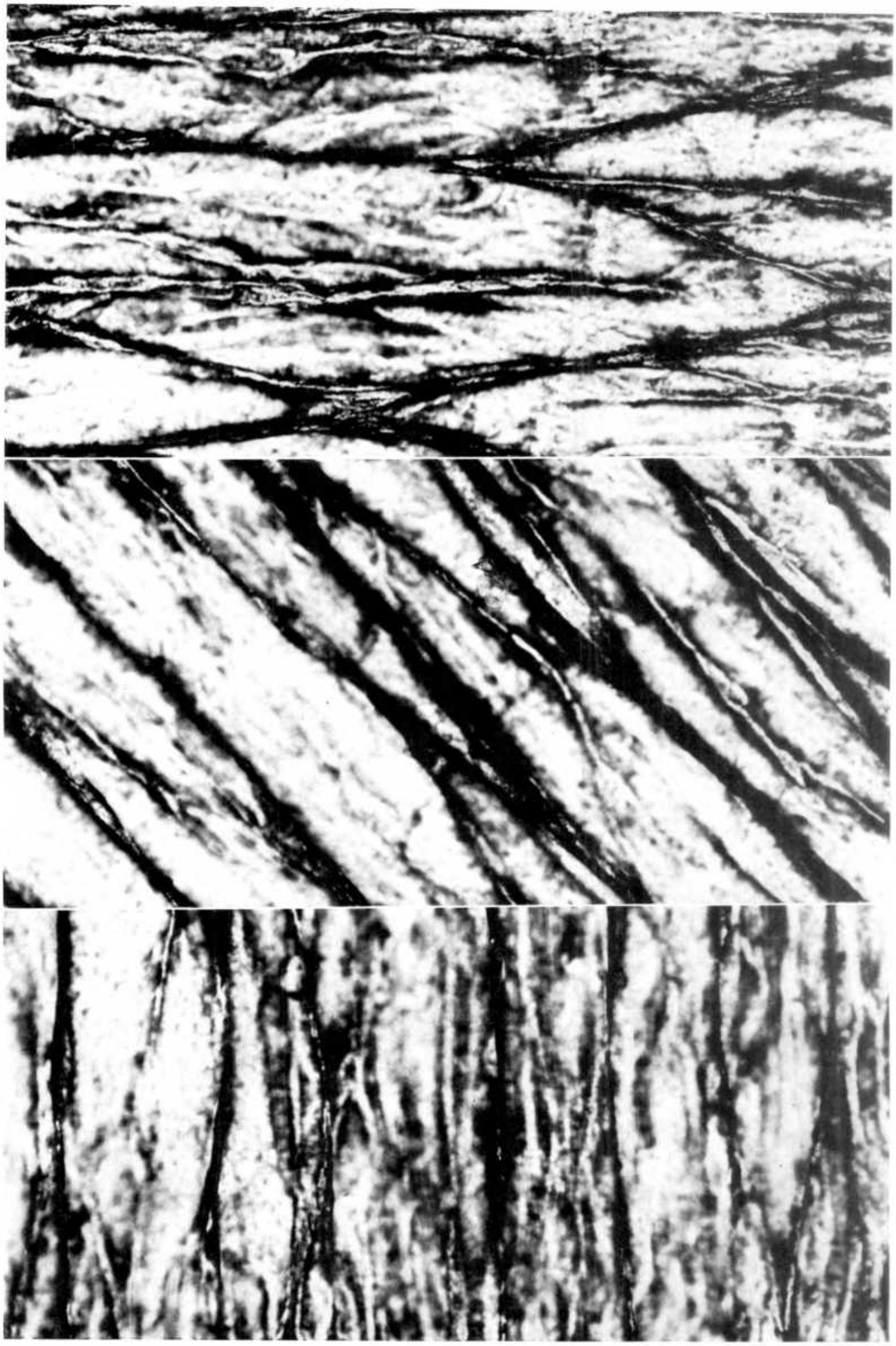

FIGURE 3. Patterns produced by uniform tension in different directions at the same site (forearm). The pattern is one of collapsed diamonds (× 50).

Special situations prevail in special anatomical sites where the particular local mechanical requirements determine the type and degree of deviation from the basic pattern. Normally the skin of the scalp is smooth and devoid of skin markings. In some instances, however, when the normal thickness of the scalp appears diminished, as in patients with alopecia areata, a delicate honeycomb pattern of regular hexagons becomes discernible, each hexagon comprising between 15 and 20 horn cells (Fig. 8). The forehead is another example of a specialized area with limited mechanical requirements; movements occur almost exclusively in one or two directions, usually at right angles to each other. The macroscopic organization is reflected at the microscopic level in which the skin surface is patterned

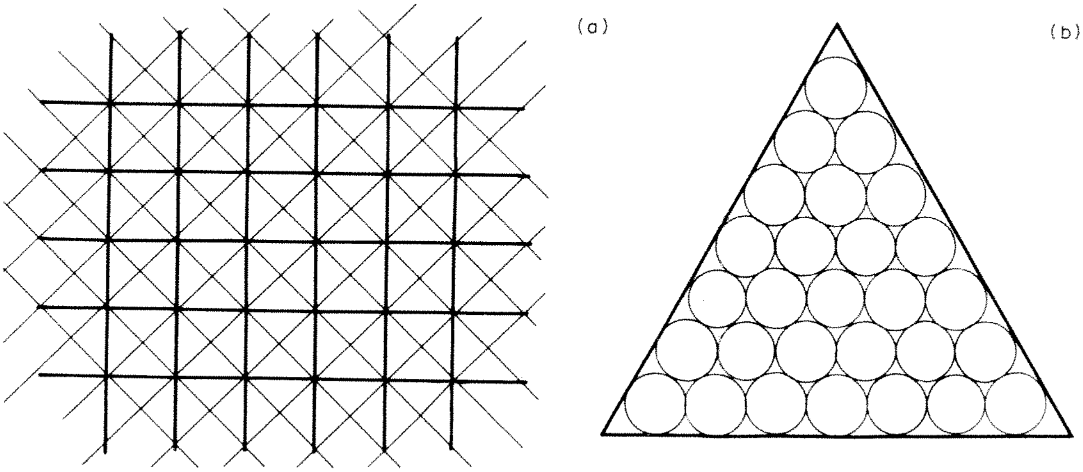

FIGURE 4. Diagram of basic skin surface structure. (a) Rectangular lattice pattern subdivided by diagonals. (b) Smaller compressible sub units filling triangles.

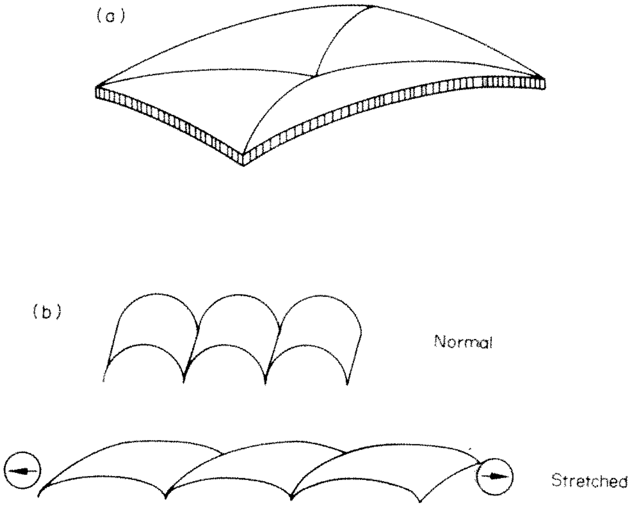

FIGURE 5. Diagram illustrating how stretching of the horny layer is achieved. (a) Model of normal unit. Note overall curving. (b) Three units in normal state. Curvature exaggerated (above) and stretched (below).

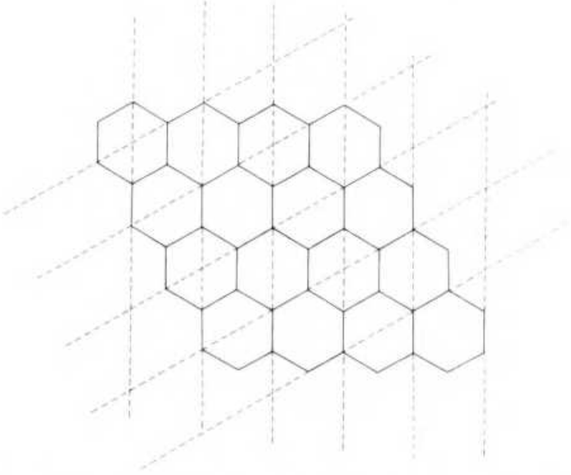

FIGURE 6. Diagram representing individual horn cells. The basic hexagonal outline is the ideal smallest structural unit for all major patterns.

only in a rudimentary fashion in parallel folds or folds which cross each other at right angles and at irregular intervals (Fig. 7).

Skin surface samples taken while skin is under tension reveal characteristic and predictable alterations from the resting pattern (Fig. 3). As soon as unilateral tension is applied the geometrical units described above resolve into a series of collapsing diamonds. The folds or creases of major and minor units deepen as they close until end-stage deformation occurs and the previously shallow creases are then approximated to form deep parallel folds. When the skin surface is sampled under circumferential

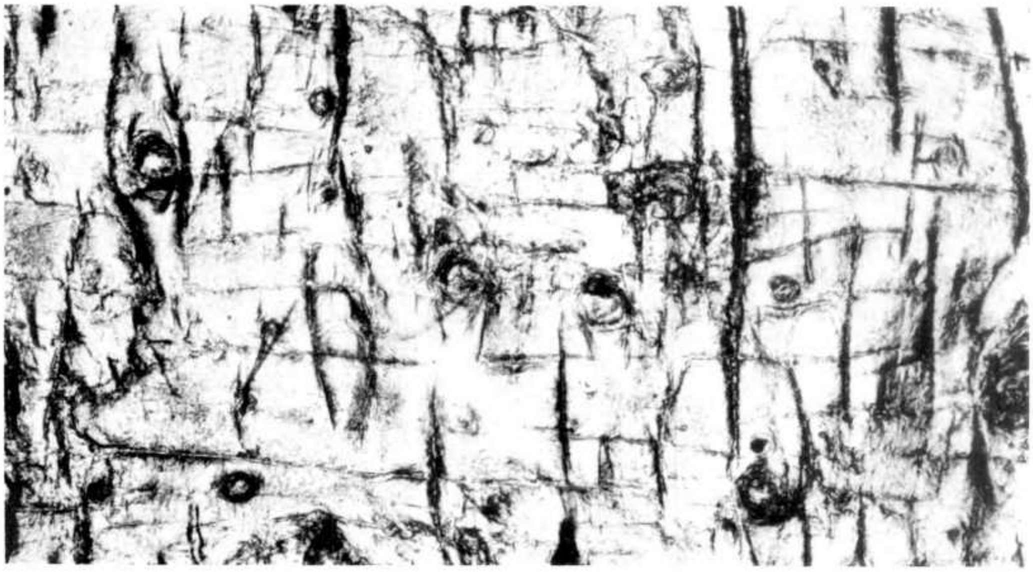

FIGURE 7. Forehead showing rudimentary pattern as compared with Fig. 1 (× 8).

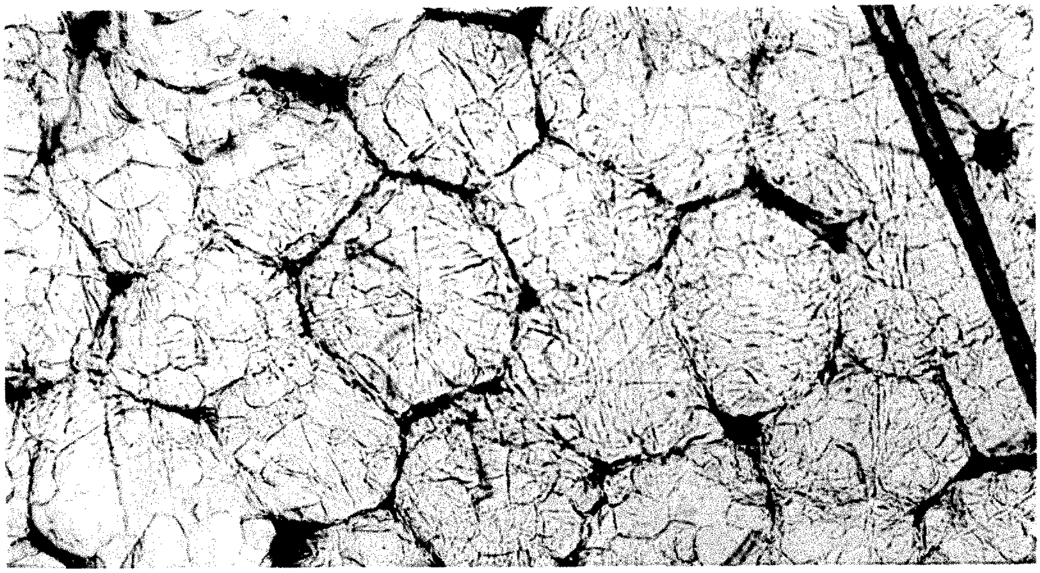

FIGURE 8. Skin surface pattern from scalp (alopecia areata). Unusual hexagonal pattern. No other skin markings present (× 8).

tension the geometric pattern is not entirely abolished but usually resolves into the simplest or ideal configuration of rectangles or squares. The finding that this pattern prevails throughout the entire thickness of the horny layer can be demonstrated by successive strippings taken from the same site.

Hairs frequently emerge at the angular junction of the folds. It is at these sites that the stratum corneum invaginates more deeply in the acrotrichium. In some instances, therefore, the hair follicle may represent a nodal point for dermo-epidermal folding. The folds as seen in the skin surface samples can also be recognized in conventional transverse tissue sections as irregular dips of the epidermis where they are not related to the ostia of hair follicles or openings of sweat ducts and are distinct from the normal relief pattern of the papillary dermis. Otherwise, no morphological clues emerge from the inspection of tissue sections as to how corneal folds are produced.

DISCUSSION

The symmetrical and orderly isometric system of rectangles, triangles, diamonds and hexagons imprinted in the skin surface is both functional and structural. The creases or indentations found in the resting skin act as pre-folds which determine the exact points of flexion and extension when the stratum corneum is deformed. The isometric geometry of the pattern itself, particularly in the form of collapsing diamonds, permits the relatively inelastic stratum corneum to accommodate to deformation or compression in almost any direction without cracking, dermo-epidermal separation or loss of intra-epithelial cohesion, provided the structural limits of the system are not exceeded. The geometry is most significantly expressed as a system of units within units in which the individual horny cell is the smallest structural unit and the rectangle or least triangle is the smallest functional unit.

The horny cell itself remains somewhat inscrutable within this simple and elegant system of interlocking geometry. What precisely determines the symmetry and hexagonal shape of the individual horny cell is not known, although it is known that if any number of identical deformable discs are pressed against eacn other they will assume a hexagonal array (Menton & Eised, 1971). Perhaps it is such a simple mechanical factor which determines their shape in non-volar epidermis. Hexagonal shapes of a variety of structures and units, both macroscopic and ultrastructural, are extensively represented throughout nature whenever resolution of a surface or volumetric array into small units of equal size is required.

Whether folding occurs at a strictly cellular level is not yet determined, but is certainly not necessary for the formation of skin folds. The size of the smallest skin folds is such that usually several horny cells bend over it. However, ultrastructural studies of horizontally sectioned horn cells reveal a somewhat ordered internal structure and microfilaments tend to be arranged in wavy and parallel spiral bundles rather than being random (Headington & Schellander, unpublished observation). What advantage, if any, these ultrastructural features provide is uncertain, because individual horn cells do not seem to alter their shape under tension. Bundles of microfilaments within an amorphous matrix may simply represent a matter of unit strength as displayed by a composite material.

Specialized function within this sytem is accommodated by either simplifying the geometric pattern through deletion or by amplifying it through multiplication. Special demands are regional and are largely based on mechanical requirements. In other words, the complexity of the surface pattern directly reflects the complexity of movements required in a certain site. This is also shown in a different way by the differences of the dermo-epidermal interface in various body areas.

What exactly determines the surface pattern, however, remains obscure. The stratum corneum is only a small part of an integrated structural and functional system that comprises the whole of the skin and further work will be required to establish the nature of the dermal–epidermal interactions that produce the skin surface pattern.

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Familial scleroderma

J.R.RENDALL* AND A.W.McKENZIE

Norfolk and Norwich Hospital, Norwich

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SUMMARY

A further instance of familial scleroderma is reported. Three sisters in a female sibship of six were involved. Possible reasons why scleroderma in families may be more common than previously supposed are discussed.

Systemic sclerosis is an uncommon disease of unknown aetiology in which a familial incidence has only rarely been reported. A review of the literature in 1969 (Burge, Perry & Stickler) revealed convincing documentation of five families and this included the two sisters whom they described. There are no diagnostic or screening serological tests, but classification on clinical grounds has suggested a spectrum of disease ranging from the relatively benign CRST syndrome (calcinosis, Raynaud's phenomenon, sclerodactylia and telangiectasia) (Carr, Heisel & Stevenson, 1965) to the more serious conditions of acrosclerosis and systemic scleroderma. We have recently studied four patients who illustrate not only the familial incidence of the condition but also different expressions of the same disease in three sisters.

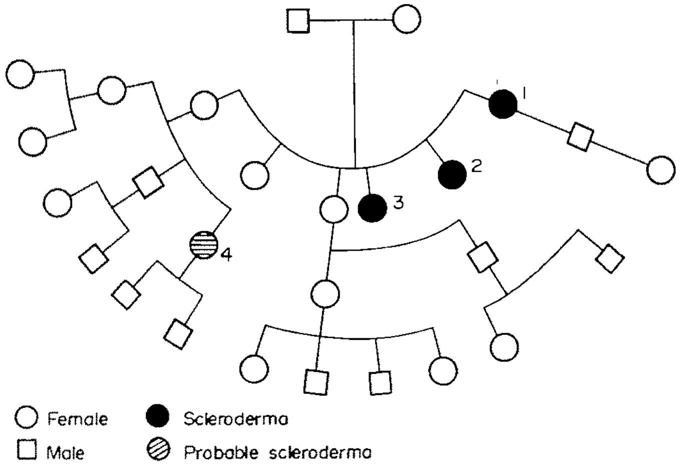

FIGURE 1.

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* Present address: The Radcliffe Infirmary, Oxford, OX2 6HE.

The pedigree is illustrated in Fig. 1 and shows the involvement of three sisters in a female sibship of six. The mother of patient 4 with probable scleroderma died in her early 70s from degenerative heart disease. The sister without children is alive and well, and the other sister died from 'old age' in her early 70s. The remainder of the family members have either been examined or their state of health ascertained from parents or affected members of the family.

CASE REPORTS

Case No. 1

Mrs E.R. This 62-year-old woman was seen in the skin clinic in October 1965 with an 18-month history of tender annular lesions on both temples. During the previous 15 years, and coinciding with Raynaud's phenomenon in the hands, red spots had appeared on the face, arms and trunk. During this time she complained of soreness of the tongue and angles of the mouth, and had suffered from intermittent diarrhoea, the stools being offensive but without blood or mucus.

Examination revealed annular necrobiotic lesions over both temples and on the outer aspect of both thighs. Flat 3–5 mm telangiectatic lesions were present on the lips, tongue, buccal mucosa, cheeks, upper arms, left breast and on the feet. Ill-defined areas of skin thickening were present on the V and nape of the neck, and on the volar aspects of the wrists. She had a smooth red tongue and angular cheilitis. These skin changes have persisted, and on follow-up marked puckering of the of the skin around the mouth has been observed, together with appearance of atrophie blanche over the medial malleoli of both ankles. No sclerosis of the digits has developed.

Investigations (1965–1973). ESR 23 mm in the 1st h. Non-quantitative Figlu test positive (1965). Schilling test showed marked decrease in vitamin B_{12} absorption. After 0·5 μCi ^{57}Cobalt and 1·0 mg vitamin B_{12}, urinary excretion of ^{57}Cobalt was 1·5% in 24 h and 0·6% between 24 and 48 h. When the test was repeated with 100 mg intrinsic factor concentrate the urinary excretion was 6% in 24 h and 1% between 24 and 48 h. Although there was a slight increase in absorption with the addition of intrinsic factor, the total 48-h figure was less than the normal for our laboratory. Faecal fat 12 g/day (3-day collection). Gastric test meal showed histamine-fast achlorhydria. Immunofluorescent antibody studies—cell nuclei positive 1 : 800 (nucleolar pattern), parietal cells positive 1 : 50. Serum immunoglobulins: IgA 315 mg/100 ml (normal range 74–280), IgE and IgM normal. Barium swallow, meal and follow-through: oesophagus and stomach normal but very rapid transit of barium through the small intestine (2 h). X-ray of extremities: no calcinosis. Normal results were obtained for haemoglobin, white blood count, rheumatoid factor, LE cells, blood urea, total serum protein fractions and glucose tolerance test.

Histology. (1) *Skin of back*: hyperkeratosis and focal parakeratosis. Fibrosis of the corium, including the subepidermal layers, extending into the subcutaneous fat. Low grade perivascular dermal inflammation. The findings are consistent with scleroderma.

(2) *Per-oral jejunal biopsy*: normal duodenojejunal mucosa.

(3) *Annular lesions of temples*: the dermis is infiltrated by histiocytes and multinucleate giant cells (some asteroid bodies), some of which are related to areas of collagen degeneration. There are foci of plasma cell and histiocytic infiltration in the fat.

Comment. We feel that the necrobiotic annules on this woman's temples are unrelated to her scleroderma. She is case no. 7 in the report of atypical (annular) necrobiosis of the face and scalp by Dowling & Wilson Jones (1967). The patient does not fit readily into any of the usual morphological classifications of acrosclerosis; she lacks the digital sclerosis and calcinosis of the CRST syndrome, and the slow tempo of her disease is not that usually associated with progressive systemic sclerosis. We feel that her malabsorption is due to scleroderma of the small intestine. She appears to have malabsorption of vitamin B_{12}, while her significant titre of parietal cell antibodies, the histamine fast achlorhydria and incomplete correction of absorption with intrinsic factor indicate that she probably has pernicious anaemia also. We are not aware of any reported association between systemic sclerosis and pernicious anaemia, although systemic sclerosis has been described with other auto-immune diseases, notably lupus erythematosus (Rowell, 1962) and auto-immune haemolytic anaemia (Chaves *et al.*, 1970). The CRST syndrome has been described in association with primary biliary cirrhosis (Reynolds *et al.*, 1971; Murray-Lyon *et al.*, 1970).

Case No. 2

Mrs E.F. This patient attended hospital initially in 1956 at the age of 53, when a 20-year history of Raynaud's phenomenon had culminated in a 6-week history of swelling and stiffness of the fingers and wrists. After an oedematous phase of 2 months, sclerosis developed in these areas and on the face, neck, chest, feet and toes. On a maintenance dosage of prednisone 10mg/day, generalized skin thickening and acrosclerosis with palmar telangiectasia developed, while heartburn, particularly on bending, became a prominent feature. Two years after the onset of her oedema and sclerosis she was admitted to hospital with left ventricular failure and anuria. There was no electrocardiographic evidence of myocardial infarction. Standard management was instituted but anuria persisted and she died at the age of 55 years.

Investigations. Barium swallow and meal demonstrated lack of normal oseophageal peristalsis and a small sliding hiatus hernia with free gastro-oesophageal reflux. X-ray of her hands showed mild osteoporosis but no soft tissue calcification. A skin biopsy from the chest was reported to show changes compatible with scleroderma. Investigation of haemoglobin, while cell count, ESR, serum proteins, blood urea, serum cholesterol and rheumatoid factors gave normal results.

Post-mortem histological findings. (1) *Heart:* small foci of ischaemic muscle damage with comparatively little inflammatory response. Foci of cellular fibrous tissue. The appearances are consistent with scleroderma.

(2) *Kidneys*: endarteritis fibrosa of medium sized arteries and intense hyaline or fibrinoid degeneration of arterioles. Glomeruli all appeared abnormal, showing thickening of capillary basement membranes and focal deposits of fibrinoid material. Adhesions between tufts and Bowman's capsules were common. Some of the tuft lobules were dilated and filled with proteinaceous material. Other tubules were atrophic. The section showed moderate autolysis and congestion. The appearances were those of hypertension and also glomerular damage which was not typically hypertensive but was entirely in keeping with a diagnosis of scleroderma.

(3) *Lungs*: confluent pneumonia with organization.

There was, unfortunately, no examination of the gastro-intestinal tract.

Comment. At no time during the patient's clinical course was she found to be hypertensive. She is considered to have suffered from acrosclerosis with oesophageal, cardiac and renal involvement.

Case No. 3

Mrs M.F. This 74-year-old woman was seen in the skin department in 1971 on account of tender and discharging swellings of the digits. Clinically and radiologically calcinosis cutis affected the fingers diffusely, together with sclerotic changes and telangiectasia. Her past history revealed that she had suffered from a cyanotic type of circulation in her hands and feet from her early 20s, and that typical Raynaud's phenomenon had developed at the age of 50. Dyspepsia was investigated in 1967 and a barium swallow and meal revealed moderate oesophageal reflux but no obvious abnormality of oesophageal motility. She was admitted to hospital in December 1972 with acute dyspnoea and severe chest pain, and died shortly afterwards from a presumed myocardial infarction. No post mortem was performed.

Investigations. A skin biopsy from a digit had been interpreted as compatible with scleroderma. The following investigations performed over several years were normal: haemoglobin, white cell count, ESR, red blood cell indices, calcium, phosphate, alkaline phosphatase, liver function tests, serum protein electrophoresis, Coombs' test, cold agglutinins, rheumatoid and LE cell tests.

Comment. This patient is considered to have suffered from classical acrosclerosis.

Case No. 4

Mrs P.D., aged 42. A niece of case no.1 residing in another part of the country had symptoms which suggested to Mrs E.R. that she suffered from the same condition as herself and her two sisters. Mrs P.D. had suffered from chilblains on the feet since childhood and in her early 20s developed Raynaud's phenomenon in the feet and hands. Between the ages of 38 and 40, ulcers developed on the toes, together with persistent pain in the left foot. Left lumbar sympathectomy to improve these ischaemic changes had been performed with marginal improvement. No definite clinical diagnosis had been made. Residence in southern England during the past 2 years had lessened her symptoms, but she had developed red spots around the mouth. When visited by one of us (J.R.R.), she showed marked acrocyanosis of the hands and feet, but no calcinosis or convincing sclerosis clinically. Eight flat telangiectatic lesions were present around the mouth. Mrs P.D.'s second son, aged 10, also complained of blueness and sensitivity to cold in the fingers, but showed no abnormality on examination. Mother and son had the following investigations performed with normal results: serum bilirubin, alkaline phosphatase, total proteins, protein electrophoresis and immuno-electrophoresis. Immunofluorescence tests for antibodies to cell nuclei, mitochondria, smooth muscle, parietal cells, thyroid microsomes and thyroid colloid were negative.

DISCUSSION

The sparse literature on familial scleroderma has been reviewed by Burge *et al.* (1969). They considered that, including their own cases, there was convincing documentation of five families (Orabona & Albano, 1958; Blanchard & Speed, 1965; McAndrew & Barnes, 1965; Burge *et al.*, 1969; Rees & Bennett, 1953.) They cited other probable instances where documentation is incomplete (Kass, Hanson & Patrick, 1966; Schimke, Kirkpatrick & Delp, 1967).

We have limited our interest to systemic scleroderma and have preferred to exclude morphoea, as occurred in the cases of Rees & Bennett (1953). Localized or generalized morphoea may result in severe secondary contractures and arthritis, but systemic involvement is only rarely seen (Curtis & Jansen, 1958).

Fleischmajer, Damiano & Nedwich (1972) have demonstrated that in scleroderma there is replacement of subcutaneous tissue by young and immature connective tissue. The circumstances which initiate these changes are unknown, however, and factors as diverse as virus infection (Kudejko, 1966) and disordered chromosomes (Housset *et al.*, 1969) have been suggested. Clinical studies all point to females being affected three or more times more commonly than males, and Burch & Rowell (1963), using large population surveys and sex and age of onset rates, have theorised on the similar pattern of lupus erythematosus, scleroderma and other auto-immune diseases. In scleroderma they postulated a mechanism of single gene X-linked inheritance requiring two somatic mutations for expression which produced the disorder mainly in females and with a peak incidence rate between the ages of 24 and 40 years.

We feel that for a number of reasons reports in the literature perhaps underestimate the incidence of familial scleroderma. The disease may produce little symptomatic disease in an individual initially and, having in females a peak incidence in the fourth decade and a mean interval of 5·4 years between Raynaud's phenomenon and the onset of scleroderma of varying degree (Rowell, 1973), may easily be overshadowed by unrelated conditions and pass unobserved. Early symptoms of peripheral intolerance to cold may persist long enough for patient adaptation to occur and for medical advice to go unsought. The disease may involve a variety of organs in related or unrelated patients, and the diversity of presenting symptoms may lead to investigation and management in different specialties. The care of patient no. 1 in our series had always been medical or dermatological, but she alerted us to the fact that her niece had symptoms similar to her own and was under the care of surgeons. The absence of a suitable serological test diagnostic of scleroderma and the lack of universally recognized criteria for diagnosis complete these difficulties. The detailed examination of relatives of affected individuals might well be a fruitful exercise and indicate the true incidence of familial scleroderma. In our own hospital practice we know of another family in which the mother has the changes of the CRST syndrome, while two daughters have less pronounced changes.

ACKNOWLEDGMENT

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Photosensitivity from nalidixic acid*

C.A.RAMSAY† AND E.OBRESHKOVA‡

Department of Photobiology, Institute of Dermatology, London E9 6BX.

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SUMMARY

Seven patients with a bullous photodermatitis from nalidixic acid are described. Bullae develop characteristically on the dorsal aspects of the feet and on the lower legs, but also elsewhere, in patients taking the drug who are exposed to a high level of ambient sunlight. At times, the clinical picture may be confused with porphyria cutanea tarda. Abnormal reactions to sunlight may continue for several months after the acute stage, whether or not the drug is stopped, but some patients may continue to take the drug if direct sunlight on the skin is avoided.

Investigations showed that long wave ultraviolet radiation appears to be responsible for the reaction and it proved possible to induce photosensitivity in three volunteers who took the drug for 1 week.

Photosensitivity induced by drugs is uncommon in the U.K., but nalidixic acid, according to the Register of Adverse Reactions (1971), is one of the drugs that causes it most often. It is also a drug which seems to produce a photodermatitis with several characteristic features. Since 1968, we have seen seven patients with such a photodermatitis and this paper reports the clinical and photobiological features.

CLINICAL DETAILS

Nalidixic acid was taken for recurrent urinary tract infection by all seven patients, all female, aged 25–55 years. Three patients were taking other drugs at the time of the skin reaction: Case 5 an oral contraceptive (Sequens, Eli Lilly & Co. Ltd); Case 6 oral prednisolone, 5 mg daily and intermittent antispasmodics (Franol, Winthrop Labs) for asthma; and Case 7 frusemide, butobarbitone and a mixture of neomycin and a poorly absorbed sulphonamide against diarrhoea which she had obtained in Spain.

The type of reaction and site of lesions

These were both characteristic. Each patient developed blisters on the dorsa of the feet and, except for Case 2, on the dorsa of the hands. Three patients (Cases 1, 2 and 5) also had blisters on the lower

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‡ Present address: Department of Dermatology, University of Sofia, Bulgaria.

TABLE 1. Clinical details of patients

Case no.	Age (years)	Sex	Duration of photosensitivity	Whether drug stopped
1	25	F	6 weeks	Yes
2	44	F	3 weeks	No
3	55	F	15 months	Yes
4	37	F	(a) 3 months (b) 3 months	No
5	29	F	2½ months	Yes
6	29	F	(a) 1–2 weeks (b) 6 weeks	Yes
7	40	F	3 months	Yes

legs. On one patient (Case 4) patchy erythema preceded the blisters.

Although the peripheral exposed parts of the limbs were so characteristically affected other areas of skin did not escape. Erythema of the face occurred in two patients (Cases 3 and 4) and a few small blisters on the forehead in another two (Cases 6 and 7). Covered parts of the body were not affected.

After the acute bullous reaction had settled, four patients (Cases 1, 2, 4 and 7) had skin fragility and two of them (Cases 1 and 2) blisters following minor trauma.

Latent period before development of rash and solar exposure required

A high level of ambient sunlight seemed to be more important than the length of time the drug had been taken. Thus one patient (Case 5) had been taking nalidixic acid for 5 weeks, went on holiday in Spain and a week later blisters appeared on the hands, feet and lower legs. Another patient (Case 2) developed blisters on the second day of her summer holiday in Malta after taking the drug for 7 months without previous trouble. By contrast another patient (Case 4) had her first bullous reaction whilst in the sun on holiday at the English coast, after a week of the drug. The bullae settled slowly over 2–3 months on returning to London, and the drug was continued during the next year with no further ill effects. One year after the first reaction, whilst still on the drug, a recurrence developed a week after solar exposure at an English seaside resort. Yet another patient (Case 6) developed her first reaction 6 weeks after stopping the drug when on summer holiday in England. She started the drug again 1 month after the first blisters had healed and developed a recurrence within 6 days, again whilst on holiday in the sun.

Duration of photosensitivity

New blisters, evidently sun induced, continued to appear for up to 3 months in all patients, whether or not the drug was stopped and at times were apparently provoked by mild trauma to the skin. For example, in one patient (Case 3) the blisters of the acute episode ceased to appear after a few days and they slowly healed 7 days after the drug was withdrawn. But she seemingly continued to have minor abnormal reactions to sunlight for more than 1 year after finishing the drug. These have taken the form of erythema and swelling of the dorsal aspects of the hands and feet after being exposed for 45 min to sunlight passing through window glass. These symptoms are now slowly regressing.

INVESTIGATIONS AND RESULTS

Biopsies

In two patients biopsies of blisters were obtained. One (Case 5) from the toe, 6 weeks after the drug was stopped, showed a blister in the superficial epidermis. Biopsies of blisters on the feet in the other patient (Case 7), 2 and 3 months after the blisters had first appeared and whilst new lesions were still developing, both showed a sub-epidermal bulla with little or no inflammatory reaction.

Qualitative tests of porphyrin metabolism

These tests (Rimington, 1958; Rimington & Cripps, 1965) were negative in blood, urine and stools in all patients except for one (Case 6) whose stool was not examined.

The absorption spectrum

A solution of nalidixic acid in 0·1 N sodium hydroxide (pH 8·4) was examined in a Beckman SP.800 recording spectrophotometer. Peak absorption was at 260 nm. At wavelengths above 290 nm there was broad absorption between approximately 320 and 340 nm (Fig. 1).

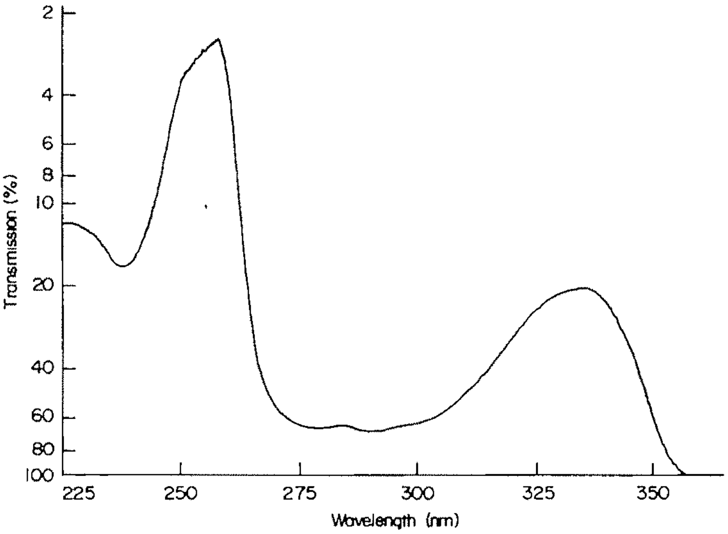

FIGURE 1. Absorption spectrum of nalidixic acid (10 μg/ml in 0·1 N sodium hydroxide, pH 8·4).

Photopatch tests

Photopatch tests were carried out on three patients (Cases 2, 4 and 5) by applying varying concentrations of the drug to skin under closed patches in duplicate. After 48 h the patches were removed, and one set of test sites was exposed for 30 min to a bank of 4 × 20 W 'black light' fluorescent tubes, approximately 30 cm from the skin. All test sites were read again at 96 h. Both irradiated and non-irradiated patch tests were negative.

Irradiation monochromator tests

These were made on the skin with a prism instrument and 2 kW xenon arc at various wavebands between 300 and 600 nm (Magnus *et al.*, 1959). It was possible to test only three patients whilst they were taking the drug (Cases 2, 4 and 6). One of these (Case 2) gave completely normal results, another (Case 4) developed delayed erythema at 340 nm on one of two test sites with a dose of 7 J/cm^2. The tests in the third patient (Case 6) were normal on the first occasion 5 weeks after stopping the drug but, when repeated after 30 g of the drug had been taken over the course of a week, they showed immediate erythema at 320 and 340 nm with doses of 1·0 and 1·5 J/cm^2 respectively.

In all other patients the irradiation tests were carried out several weeks to several months after stopping the drug. In two (Cases 3 and 7) clinical photosensitivity was still present when the tests were performed. Case 3 showed delayed papular type responses at 307·5 and 320 nm but was other, wise normal; Case 7 showed delayed erythema at one of two sites irradiated at 360 nm. The remaining two patients (Cases 1 and 5) had normal results but tests were performed when the skin was normal and after the drug had been stopped.

Tests on volunteers

In addition to the narrow waveband tests with the monochromator described above, a broad band long wave UVR source was also used. A 5 cm^2, 1 mm thick dichroic mirror (Balzer) in the xenon arc beam was used to separate the UVR, which it reflects at 45°, from the visible and infra red radiation, which it transmits. The reflected UVR was then passed through nickel oxide and WG 345 filters and focused on to the skin with a crown glass lens (diameter 6·4 cm, focal length 11·75 cm). The skin was thus irradiated with a band of UVR extending from about 320 to 400 nm. A total dose of 45 J/cm^2 produced no reaction, apart from occasional, transient pigment darkening, in twenty normal subjects

One volunteer who showed no reaction to the long wave UVR source took nalidixic acid (4 g daily) for 1 week. At the end of this time irradiation with the long wave UVR source (45 J/cm^2) produced immediate and delayed erythema. Repeated at intervals after the drug had been stopped positive results occurred up to 6 months, after which the test became negative.

Two other volunteers took nalidixic acid in a dose of 4 g daily and were tested with monochromatic radiation at 300, 320, 340 and 360 nm before and after taking the drug for 1 week. Transient immediate erythema at 320 and 360 nm was seen after the drug in both subjects and one also showed this reaction at 340 nm, but tests were otherwise normal.

Unfortunately the long wave UVR source described above was not available for these volunteers until 2 weeks after the drug had been taken. At this time, however, a dose of longwave UVR of 45 J/cm^2 produced delayed erythema appearing at 6–8 h after irradiation and persisting for approximately 2 days Similar results were obtained in both volunteers at intervals for the next 6 months after which the tests became negative.

Animal studies

Hairless mice were used. A solution of nalidixic acid in 0·1 N NaOH was diluted to 1 mg/ml with 0·9% NaCl, the pH adjusted to 7–7·3 with 0·1 N HCl and the solution sterilized by millipore filtration.

In one group of thirteen mice 200 μg of drug were injected intradermally and 20 mins later the site was irradiated with the long wave UVR source or with monochromatic UVR at 330, 360, 380 and 400 nm (the doses given were 10–40 J/cm^2). The reaction was assessed after the intraperitoneal injection of 0·2 ml of a solution of Coomassie Blue (40 mg/ml) immediately and at intervals for 48 h after irradiation.

Another group of six mice was injected intraperitoneally with 300 μg of the drug daily for 7 days and then the flank skin irradiated with the longwave UVR source (45 J/cm^2). The reaction was again assessed by means of intraperitoneal Coomassie Blue.

The results of the irradiation tests with the drug by both routes were consistently negative.

DISCUSSION

The bullous type of photodermatitis which occurs in patients taking nalidixic acid usually presents a very characteristic clinical picture. The patient is almost invariably female and develops the reaction after exposure to excessive amounts of sunlight. A period varying from a few days up to a year may elapse between starting the drug and the development of the rash, the length of the apparent delay period being determined seemingly by the opportunity for exposure to sunlight. In all of our patients the reaction developed whilst on holiday in the summer, either in this country or abroad. Although the eruption may occur on any exposed site, there is a predilection for the lower legs and feet.

One important differential diagnosis to be considered in some patients is porphyria cutanea tarda (PCT), especially when lesions lie on the dorsal aspects of the hands. The history of fragility of the skin and blisters after minor trauma may also cause confusion with PCT. However, we have never seen milia, although common in PCT, in nalidixic acid photosensitivity. There is no reason to think that nalidixic acid is porphyrinogenic as porphyrin excretion was normal in all of our patients. This confirms the findings of Birkett, Garretts & Stevenson (1969) who investigated five patients for porphyrin excretion and found it normal.

Another characteristic feature of the nalidixic acid reaction is the persistence of the photosensitivity often for 2–3 months after the drug has been stopped; new blisters continue to appear though perhaps some are induced by trauma. In one of our patients mild photosensitivity had persisted for more than 1 year after an acute episode but eventually resolved.

The pattern of the morphology, distribution and natural history in our patients is very similar to that reported by Zelickson (1964); Guilaine (1965); Susskind & Lyell (1965); Burry & Crosby (1966); Baes (1968) and Birkett, Garretts & Stevenson, (1969). In those instances where skin biopsy was performed a sub-epidermal blister was found (Baes, 1968; Birkett *et al.*, 1969). This was seen in one of our patients, but in another the blister was intra-epidermal, probably due to regeneration of the blister base.

The mechanism of the photosensitivity remains unsolved. The drug is rapidly absorbed and almost entirely excreted by the kidneys. Tissue levels, estimated after long term administration in animals, gave little evidence of accumulation, though unfortunately skin was not examined (McChesney *et al.*, 1964). Alexander & Forman (1971) reported their experience with the lymphocyte transformation test in patients with reactions to nalidixic acid, the test being negative in those with the bullous type of response.

The negative photopatch tests in the three of our patients examined are similar to the negative results reported by Baes (1968) in twelve normal volunteers. He used 290–320 nm from a water cooled mercury arc and long wave UVR from the same source with a Schott WG 5 filter. In the same way, Birkett *et al.* (1969) were unable to induce photosensitivity in seven normal persons with oral nalidixic acid using a medium pressure mercury vapour lamp for irradiation. The minimal erythema dose was determined before and 2 weeks after taking the drug. However, these authors did not use filtered radiation and it is possible that only small amounts of longwave UVR were administered. If the absorption spectrum of the drug (Fig. 1) is in any way related to the action spectrum, then longwave UVR would be expected to be important. Although our animal studies were unsuccessful, we were able to induce abnormal photosensitivity with the drug in human volunteers.

The transient immediate erythema, which occurred with monochromatic radiation between 320 and 360 nm, must be interpreted with caution. That this did not develop before the drug was taken may lead one to suspect that the reaction was significant, but normal skin may not uncommonly develop transient immediate erythema at these wavelengths. However, the results with the broad band UVR source were more definite. One volunteer continued to give persistent delayed erythematous reactions with this source for 6 months after taking the drug, although the reaction was negative before the drug was started. Similarly, two other volunteers gave positive reactions for the same length of time after the drug. These two volunteers were not tested with this source before the drug was taken, but the fact that the test eventually became negative suggests that the drug was responsible. The results obtained with monochromatic radiation in our patients must be interpreted with care, because when abnormal reactions developed (immediate or delayed erythema) they were not always reproducible. The indefinite results obtained with monochromatic UVR, compared with the more clear cut results with the broad band longwave UVR source, are probably due to the much larger total dose that can be given with the latter.

The evidence supporting this bullous reaction as a photodermatosis due to nalidixic acid on clinical grounds is circumstantial but strong. Our experience suggests that patients may continue to take the drug providing they avoid direct sunlight on the skin. It would probably also be wise to give similar advice to all patients taking the drug until further evidence is forthcoming as to the mechanism underlying the reaction.

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Decreased membrane-bound ATP-hydrolytic activity in psoriatic epidermis*

G.MAHRLE† AND C.E.ORFANOS

Department of Dermatology, University of Cologne, Germany

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SUMMARY

Previous studies from our laboratory suggested that architectural alterations of the cell membranes may have a major significance in the pathogenesis of psoriasis. Consequently, the membrane-bound Mg^{++} activated ATP-hydrolytic activity was investigated in normal and psoriatic epidermis under the electron microscope.

The present study revealed that, in contrast to normal epidermis, only minimal ATP-hydrolytic activity is present on the cell membranes of psoriatic keratinocytes. This finding may reflect either a diminished attachment of substrate on the altered cell surface or an enzyme defect of the cell membrane. In both cases the reduced interaction between ATP and membrane-bound ATP-hydrolysing enzymes represents a functional membrane disorder of the psoriatic keratinocyte, presumably resulting in an insufficient utilization of ATP for active transport mechanisms on the cell surface.

It has been repeatedly reported that membrane-bound enzymes and the architecture of the cell membrane itself may play an important role in cell metabolism and cell growth (Robinson, Butcher & Sutherland, 1968; Burger & Noonan, 1970; Heidrick & Ryan, 1971). In human epidermis the epinephrine-cyclic AMP cascade has been proposed as a growth control mechanism, mediated by the membrane-bound activity of adenyl cyclase (Voorhees & Duell, 1971; Voorhees, Duell & Kelsey, 1972).

In previous studies we reported that alterations of the plasma membranes occur in psoriatic keratinocytes (Orfanos, Christenhuss & Mahrle, 1969; Orfanos, 1971) and speculated that the derangement of the architecture of the membranes may have a major significance in the pathogenesis of the disease (Orfanos *et al.*, 1973). If this is the case, the lack of beta-receptors on the altered cell surface would cause the reduced activity of the membrane-bound adenyl cyclase, the decreased amounts of cyclic AMP found in psoriatic epidermis (Voorhees, *et al.*, 1972), and consequently, the increased mitotic rate of the psoriatic basal cells.

In regard to this concept we assumed that such structural alterations may also involve the membrane-bound ATPase activity, as has been found in epithelial neoplasias (Sugar, Csuka & Toth, 1968). In this paper we report our findings on the ultrastructural localization and on the activity of ATP-hydrolytic enzymes in normal and in psoriatic human epidermis.

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† Reprint requests to: Dr G. Mahrle, University Skin Clinic, 5 Koln-Lindenthal, West Germany.

MATERIALS AND METHODS

Four-millimetre punch biopsies from untreated psoriatic lesions of two male patients and from the normal skin of two healthy male volunteers were obtained under local anaesthesia with 1% Scandicain. The skin specimens were prefixed at 4°C in a fixative consisting of 4% paraformaldehyde, 2·5% glutaraldehyde and 0·05% $CaCl_2$, buffered with cacodylate buffer at pH 7·2, for 4 h. The specimens were cut into 1·0 mm thick pieces during prefixation and were sliced into approximately 0·1 mm thick flakes after prefixation. The tissue flakes were rinsed in cold cacodylate buffer (pH 7·2), then incubated in a modified Wachstein–Meisel medium as described by Farquhar & Palade (1966), (5 mM ATP*, 5 mM $MgSO_4$, 100 mM NaCl, 30 mM KCL, 2·4 mM $Pb(NO_3)_2$ and 80 mM Tris-maleate buffer, pH 7·2) for 90 min. at 37°C, then fixed in 1% osmic acid, buffered with acetate–veronal buffer pH 7·2, dehydrated in rising concentrations of acetone and finally embedded in Araldite.

Control sections were incubated in the same medium without ATP. Ultrathin sections were obtained and examined with an electron microscope. Some sections were stained with uranyl acetate.

RESULTS

Normal epidermis

The most striking finding in normal epidermis was the presence of electron dense reaction products in the upper cell layers of the stratum Malpighii and in the stratum granulosum, where electron dense deposits of lead phosphate outlined numerous keratinocytes and partly filled the intercellular spaces (Fig. 1). Such deposits were more sparse or absent at the transitional zone to the stratum corneum and between the horny cells. They were decreased towards the lower layers of the stratum Malpighii, where electron dense granular material faintly marked the cell membranes. The desmosomes showed little or no reaction product.

A distinct positive reaction was also found in the stratum germinativum of normal epidermis (Fig. 2). However, at this level only the basal cell membranes facing the dermis were clearly stained and conglomerates of lead phosphate were found at the inner leaflet, resembling densely stained pinocytotic vesicles (Fig. 3a). In contrast to the findings in the granular layer no reaction product was found in the intercellular spaces of the basal layer. Also the space between the basal cell membrane and the basal lamina itself showed no reaction product at all. The cell membranes of the melanocytes and the Langerhans' cells were stained to some degree. Furthermore, the nucleoli of the keratinocytes and of the dendritic cells and their centrosomes (Fig. 3b) were densely stained. In the mitochondria of all epidermal cells distinct granular deposits were present on their cristae (Fig. 3c).

Interestingly enough, some keratohyalin granules of the stratum granulosum, particularly those of the lower cell layer, were loaded with abundant fine deposits whereas the granules of the uppermost layer were regularly free of reaction product (Fig. 1).

Psoriatic epidermis

In specimens of psoriatic epidermis a similar reaction was seen on the cell membranes and in the intercellular spaces (Fig. 4). However, the dense deposits failed to form a continuous intercellular layer and large areas of the cell membranes remained unstained. Particularly, the intercellular spaces

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* Adenosin-5′-triphosphorsäure Dinatriumsalz, Merck, Darmstadt, Germany.

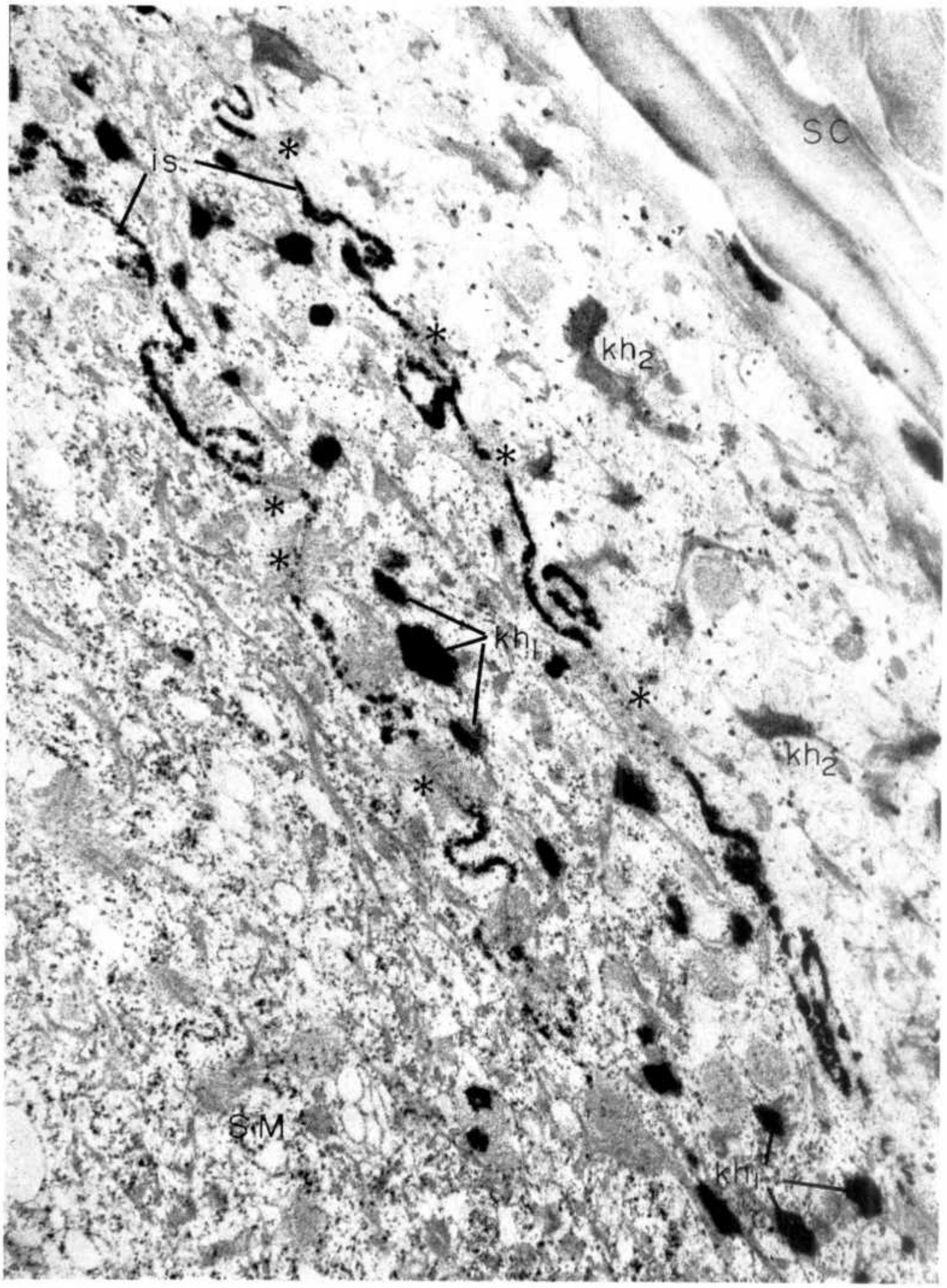

FIGURE 1. In normal epidermis, electron dense reaction products outline the keratinocytes and partly fill the intercellular spaces (is), especially in the stratum granulosum. The desmosomes show little or no reaction product (*). Small keratohyalin granules in the lower layers of the stratum granulosum (kh_1) are markedly stained compared to those in the upper most cell layer (kh_2). SC, stratum corneum; SM, stratum Malpighii (× 28,000).

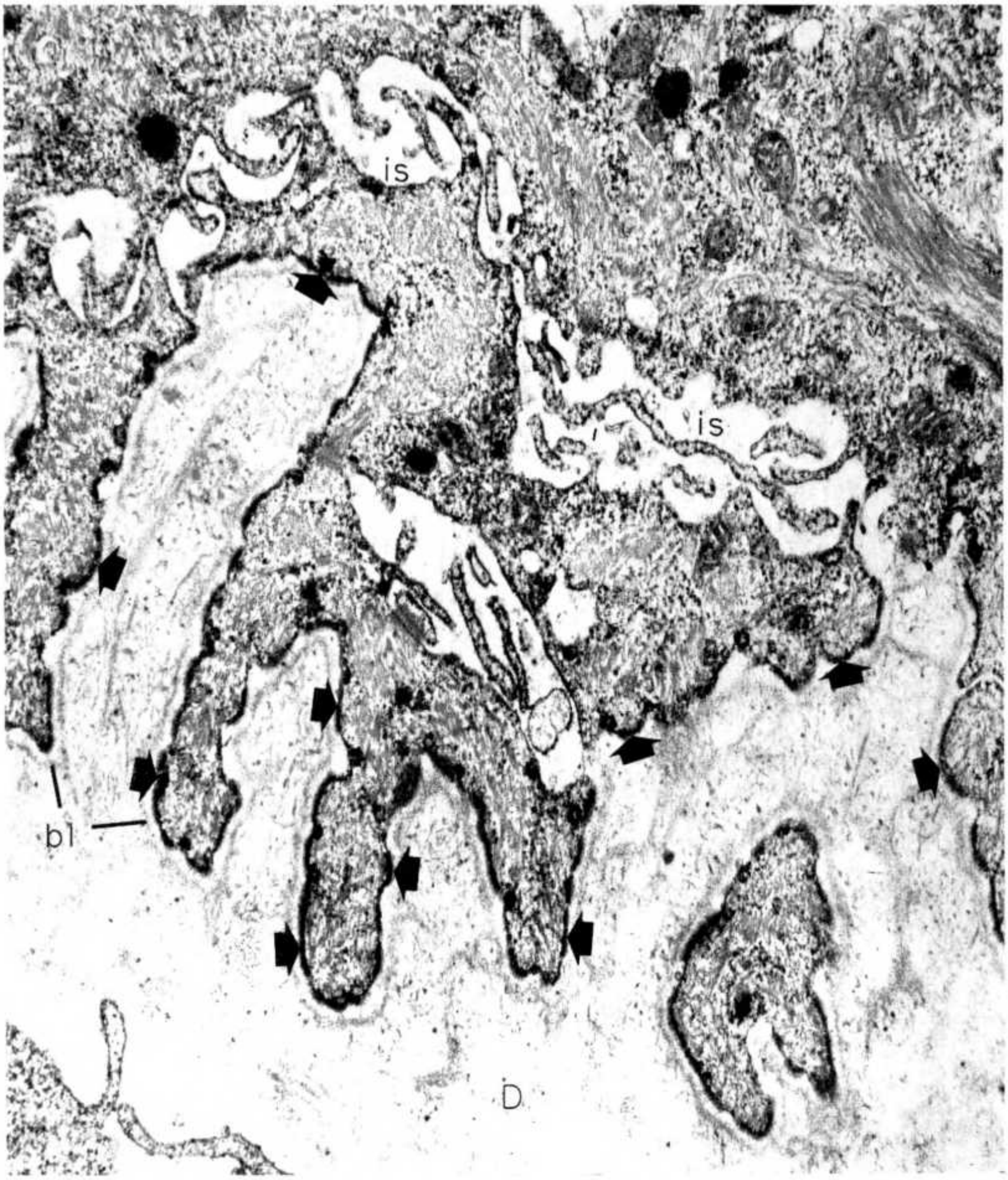

FIGURE 2. The basal cell membranes facing the dermis (arrows) are clearly stained, whereas the intercellular spaces (is) of the basal layer show no reaction product at all. bl, unstained basal lamina; D, upper dermis (× 24,500).

of the granular layer were unstained and in the basal cell layer only little reaction product was seen on the cell membranes facing the dermis, although some pinocytotic vesicles still contained electron dense material.

On the other hand, small keratohyalin granules (Fig. 5), if present, andt he nucleoli of the proliferating psoriatic keratinocytes, were heavily loaded with reaction product (Fig. 6). The keratinosomes

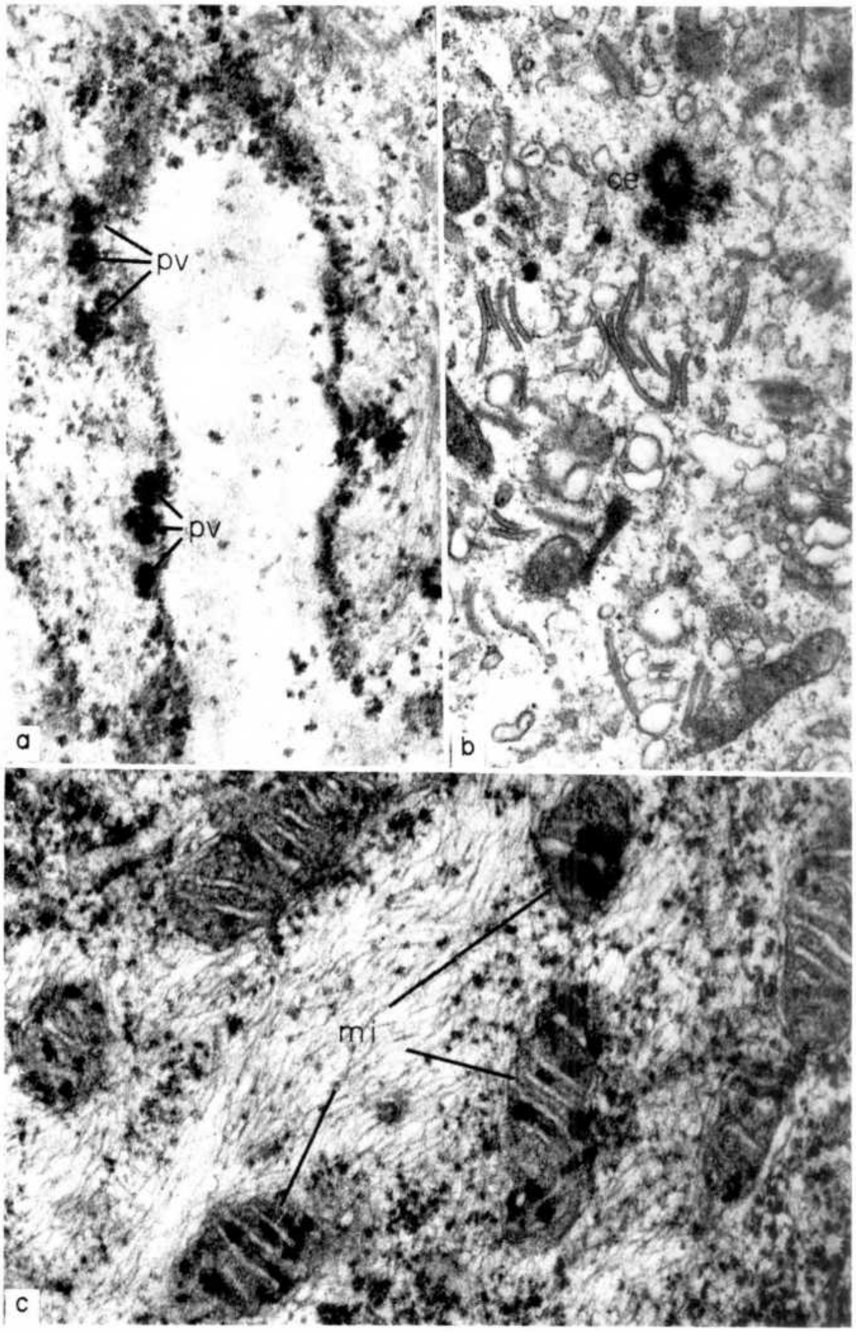

FIGURE 3. (a) Deposits of lead phosphate (pv) occur at the inner leaflet of the basal cell membrane facing the dermis, resembling densely stained pinocytotic vesicles (× 59,850). (b) The centrosome (ce) of a Langerhans' cell is densely stained (× 27,000). (c) In most mitochondria (mi) distinct granular deposits are present on their cristae (× 59,850).

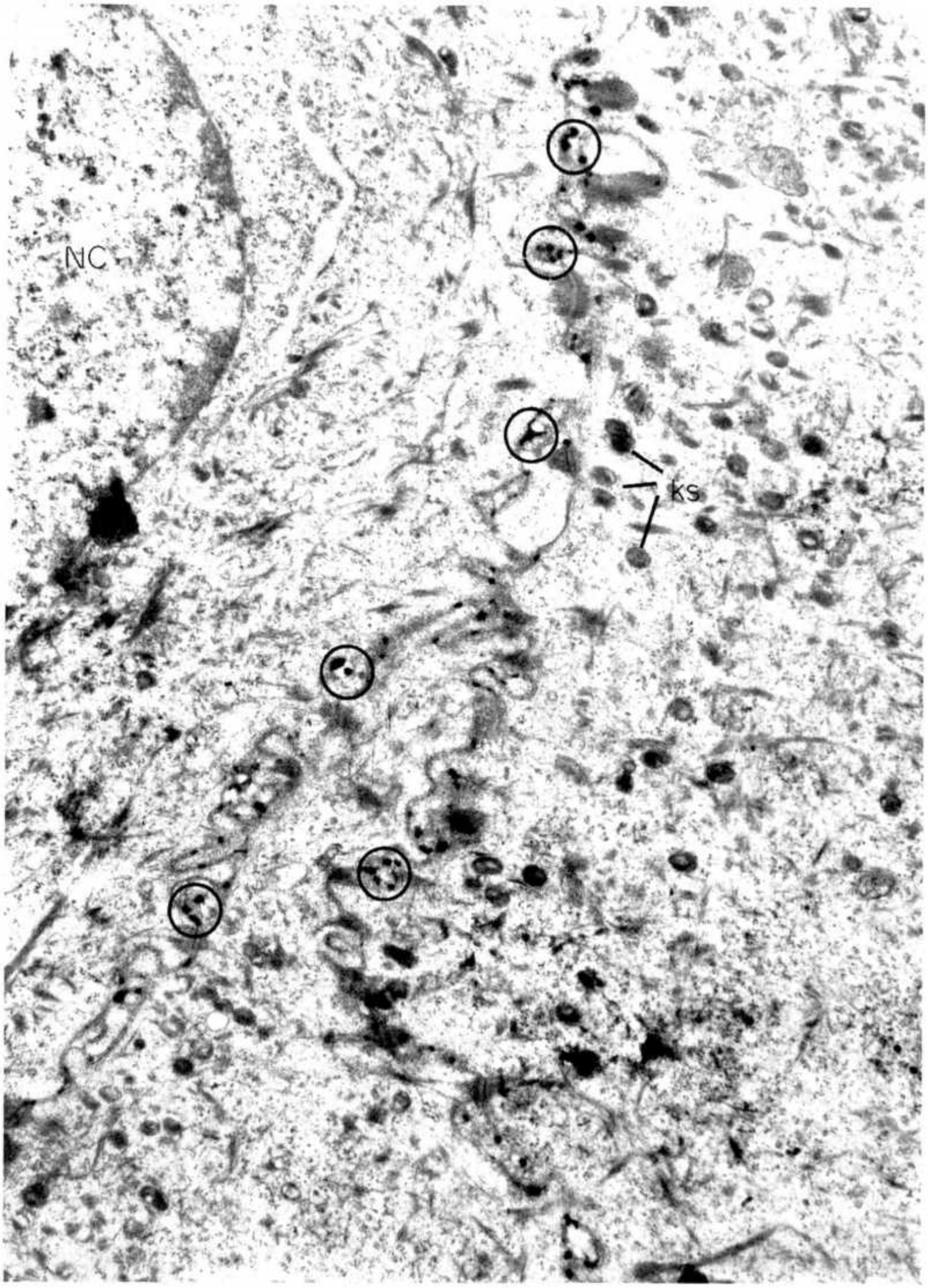

FIGURE 4. In psoriatic epidermis small granular deposits are present on the cell membranes and in the intercellular spaces (encircled). NC, nucleus; ks, keratinosomes (× 21,000).

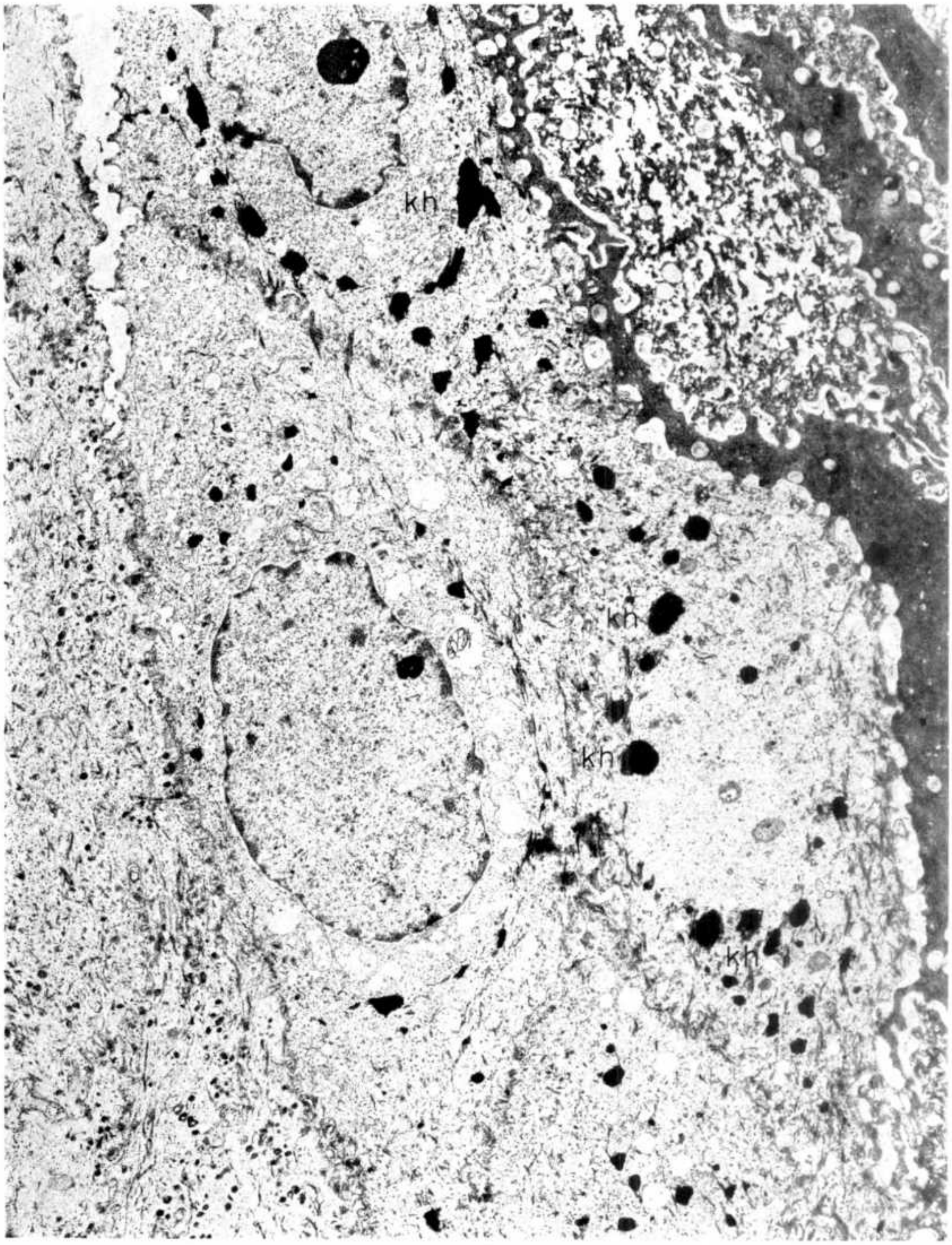

FIGURE 5. The intercellular spaces of the psoriatic stratum granulosum are free of any reaction product (compare Fig. 1). kh, densely stained keratohyalin granules (× 7000).

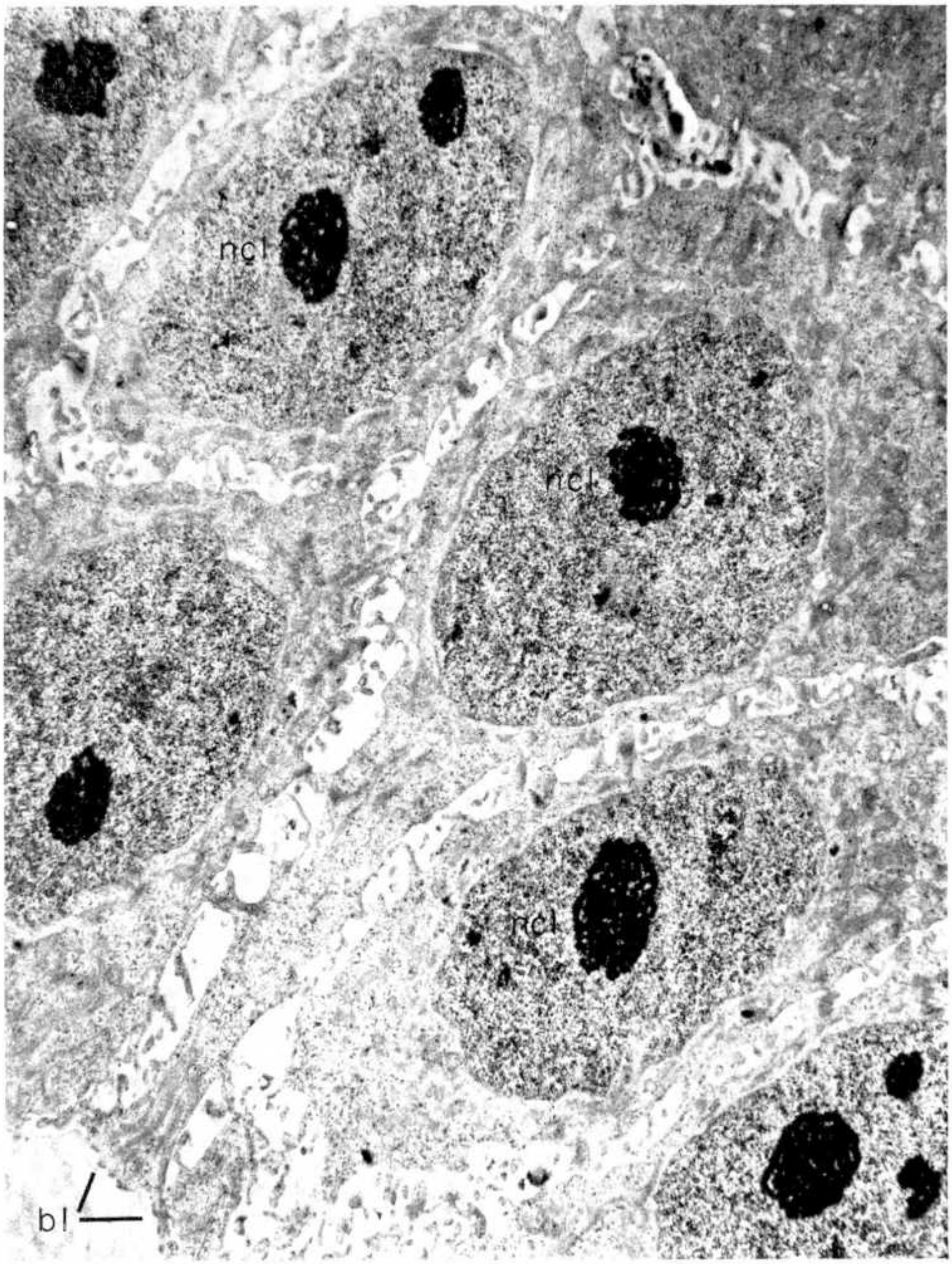

FIGURE 6. The nucleoli (ncl) are markedly stained in psoriatic epidermis, similar to normal epidermis. bl, unstained basal lamina (× 7500).

contained no deposits. Overall, the deposition of lead phosphate was clearly diminished in the intercellular spaces and on the cell surfaces in psoriatic epidermis.

Controls

In control specimens of normal (Fig. 7a) and psoriatic epidermis (Fig. 7b) incubated in the same medium lacking ATP, no electron dense reaction product was detected on the cell membranes, the

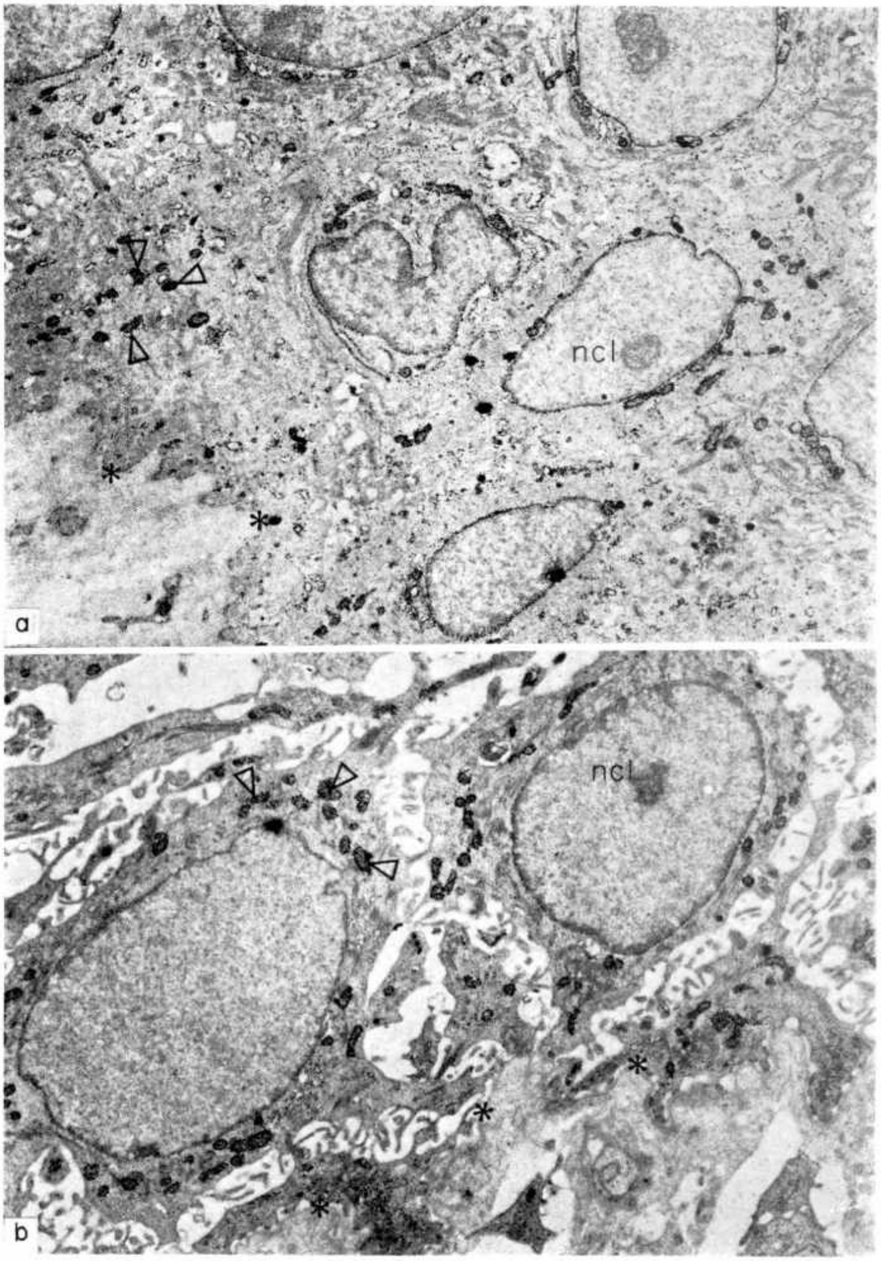

FIGURE 7. Control sections of normal (a) and psoriatic epidermis (b) reveal non-specific staining of the mitochondria (arrowed) and of the perinuclear space. No deposits are found in the nucleoli (ncl) and on the cell membranes. Dermo-epidermal interface *, (a, × 7000; b, × 6300).

pinocytotic vesicles, the nucleoli, the keratohyalin granules, or the centrosomes of the epidermal cells. It seemed, therefore, that the reaction product seen in incubated sections was indeed due to the activity of ATP-hydrolysing enzymes. On the contrary, in mitochondria, melanosomes and the perinuclear space fine granular deposits were seen in control specimens, which, therefore, were considered to be partly non-specific.

DISCUSSION

Normal epidermis

Light-microscopic observations on the activity and the localization of ATP-hydrolysing enzymes in normal human epidermis have been contradictory. Bradshaw *et al.* (1963) found the greatest enzymatic activity in the mitochondria of the basal cells and no activity in the granular layer. Jarrett & Riley (1963) reported a marked band of activity in the granular layer, which, according to Cormane & Kalsbeek (1963), was mainly confined to the keratohyalin granules. Other authors have reported a positive staining of the dendritic epidermal cells (Wolff & Winkelmann, 1967; Muller, Winkelmann & Vandersteen, 1971).

Obviously, most of these contradictory findings may be ascribed to differences of technique. It seems, however, that ultrastructural methods are more suitable for the localization of enzymatic activity of low intensity, such as ATP-hydrolysis in tissue. In 1966 Farquhar & Palade clearly demonstrated, at the ultrastructural level, that ATP-hydrolysing enzymes are localized on the cell membranes and in the intercellular spaces of all epidermal cells in amphibian epidermis. Similarly, Zelickson & Mottaz (1968) showed under the electron microscope, that similar findings may be observed in human epidermis and that ATPase-staining should not be used as a means of localizing Langerhans' cells.

The findings reported in the present study confirm the quoted electron microscopic reports on amphibian and on normal human epidermis. According to our observations Mg^{++} activated ATP-hydrolysing enzymes are almost entirely localized in the plasma membranes of all types of epidermal cells, particularly in the intercellular spaces of the granular layer. Moreover, this study revealed that the centrosomes (Abels, Jokelainen & Verhage, 1972) and the nucleoli of the epidermal cells also possess enzymatic activity. The accumulations of electron dense material in the mitochondria of the epidermal cells may be partly non-specific when using this technique.

Additionally, the findings of the present study suggest that ATP hydrolysis occurs during the first steps of keratohyalin formation since the small immature keratohyalin granules, in normal and psoriatic epidermis likewise, were clearly stained. This indicates that keratohyalin formation is an energy-dependent metabolic process requiring ATP hydrolytic enzymes.

It is difficult to give an explanation for the intensive staining of the basal cell plasma membrane facing the dermo-epidermal junction. Since we used a prolonged incubation time (90 instead of 45 min) (Farquhar & Palade, 1966; Zelickson & Mottaz, 1968) this finding may correspond to a low intensity ATPase, strictly limited to the cell membranes at the dermo-epidermal interface. The accumulation of the reaction product in the pinocytotic vesicles of the basal cells underlines their functional significance, and suggests that the postulated model of an 'outward facing membrane' (Koefoed-Johnsen & Ussing, 1958), freely permeable to Na^+ but impermeable to K^+, may exist in human epidermis. On the other hand, the heavy deposition of lead phosphate in the intercellular spaces of the stratum granulosum may be due partly to the activity of ATP-hydrolysing enzymes other than membrane-bound ATPase, particularly phosphomono- and diesterases. Such enzymes may be involved in the degradation processes taking place at the plasma membranes and the epidermal intercellular spaces, when the keratinocytes become horny cells.

Psoriatic epidermis

The evidence presented in this study shows that ATP hydrolytic activity is markedly reduced on the cell membranes of psoriatic epidermis. Since the entire capacity of psoriatic tissue to hydrolyse ATP is not significantly altered (Hammar, 1973b), it seems likely that only the membrane-bound ATP-hydrolytic activity is decreased in psoriasis. The single psoriatic keratinocyte, therefore, does not utilize the available energy pool for transport mechanisms on its surface. The increased amount of ATP which has been detected in psoriatic keratinocytes (Hammar, 1973a) is an energy pool for the increased metabolism in the cytoplasm of the cells rather than for membrane-bound cell activity.

It remains open to question as to whether the decreased ATP hydrolysis in psoriasis is due to a decreased activity of membrane-bound ATP-hydrolysing enzymes or to a limited access of substrate on the cell surface. One may argue that the loss of glycocalyx in psoriasis represents either a loss of cell surface ATP-receptors or an enzyme defect on the cell membrane. In both cases the reduced interaction between ATP and ATP-hydrolysing enzymes reflects a disturbed membrane function of the psoriatic cell. However, ATP does penetrate into the psoriatic keratinocytes, since the nucleoli were clearly stained.

Similarly, in methylcholanthrene induced mouse squamous cell carcinoma 'ATPase activity' was present on the surface of only a few tumour cells and was less intense (Sugar *et al.*, 1968). In accordance with our findings in psoriasis this may also be related to the loss of glycocalyx and of contact inhibition of growth in malignant transformed cells (Heidrick & Ryan, 1971). In other words, the psoriatic keratinocyte seems to be a rapidly growing but isolated cell, resembling tumour cells.

In conclusion, our findings show that the alteration of the membrane architecture of the psoriatic keratinocyte involves the membrane-bound ATPase function, too, as is assumed for membrane-bound adenyl cyclase. Whether there is a relationship between these two membrane enzymes and their decreased activity in psoriasis is subject to further investigations. Both enzymes utilize the same substrate and may have similar receptors on the cell membrane.

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Macromolecular metabolism in hyperplastic epidermal disease—a radio-autographic study

KENNETH A.ARNDT AND IRWIN M.FREEDBERG

Department of Dermatology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts, U.S.A.

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SUMMARY

Radio-autographic techniques have been applied to the study of three hyperplastic human epidermal diseases; psoriasis, seborrhoeic keratoses and Bowen's disease. In each, a different alteration of nucleic acid and protein synthesis has been found when the abnormal tissue is compared to clinically normal contiguous skin. Psoriasis was characterized by stimulation of DNA, RNA and protein synthesis, while in mature seborrhoeic keratoses DNA synthesis was decreased at the same time as RNA and protein synthesis were increased. In Bowen's disease DNA and protein synthesis were enhanced, but the number of cells involved in RNA synthesis was decreased.

Similar methods have been applied to psoriatic epidermis during treatment with vitamin A acid ointment. The medication resulted in inhibiton of DNA synthesis at a time when RNA and protein synthesis were enhanced.

There are many common hyperplastic epidermal diseases in man, including psoriasis, seborrhoeic keratoses and neoplastic processes such as Bowen's disease, whose clinical course and histopathology are well described but whose pathophysiology remains undefined. Consequently, diagnosis of these processes remains morphological and therapy empirical. During the recent past *in vitro* biochemical techniques for the precise study of protein and nucleic acid metabolism in model epithelial systems have been described (Freedberg, 1972). Although application of these techniques to diseased human epidermis may eventually lead to direct information concerning hyperplastic epidermal disease, the techniques are not yet applicable to such studies. High resolution radio-autography, on the other hand, is applicable to such problems and has been used in several laboratories to obtain information concerning psoriasis and cutaneous tumours (Weinstein & Frost, 1969). The studies reported in this communication were undertaken with similar radio-autographic methods and were designed to evaluate the abnormalities of epidermal DNA, RNA and protein synthesis in several types of hyperplastic and neoplastic disease.

MATERIALS AND METHODS

Adult patients, from whom informed consent was obtained, were selected from the clinics and in-patient population of the Beth Israel Hospital. The experiments were performed under licence from the United States Atomic Energy Commission and the protocol used is graphically summarized in Fig. 1. One tenth of a millilitre of sterile saline containing 5 μCi of sterile, pyrogen-free thymidine (methyl-H^3; 6·7 Ci/mM), uridine (5-H^3, 28 Ci/mM) or L-leucine (4·5-H^3; 50 Ci/mM), obtained from New England Nuclear Corporation, Boston, Massachusetts, was injected intradermally at the junction between normal tissue and the lesion under study. The weal formed was outlined with gentian violet. One hour following injection an excisional biopsy of the normal and diseased tissue encompassing the area of injection was performed and the tissues were processed for radio-autography according to standard techniques (Rogers, 1967). In the present communication four patients with psoriasis, four with seborrhoeic keratoses and one with Bowen's disease are included. In each patient, three excisional biopsies were obtained.

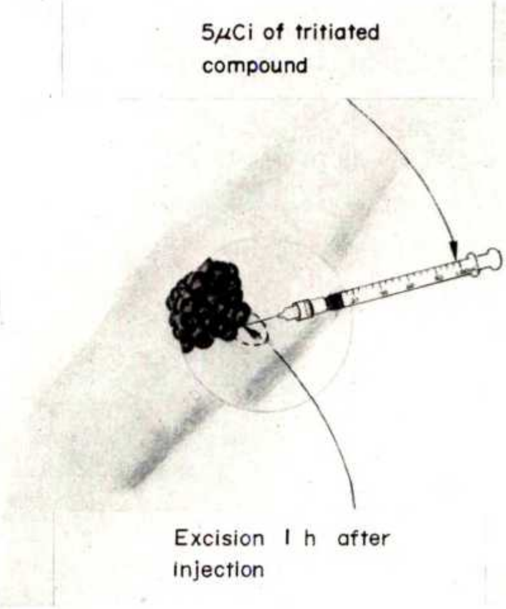

FIGURE 1. Schematic drawing of the experimental technique. 5 μCi of the appropriate precursor were injected intradermally at the junction of the normal skin and the lesion. One hour later, an ellipse including normal and pathological tissue was removed and subjected to the radio-autographic procedures detailed in the text.

Histologic sections of 6 μm were dipped in Kodak nuclear tracking emulsion (NTB-2) and incubated at 4°C for various periods of time. Leucine-labelled specimens used to evaluate protein synthesis were exposed for 3–4 days; uridine-labelled specimens used to evaluate RNA synthesis were exposed for 4–5 days; and thymidine-labelled specimens used to evaluate DNA synthesis were exposed for 7–10 days. Grain development at these times was adequate for counting. Representative uridine-labelled sections were incubated with bovine pancreatic ribonuclease (Sigma Chemical Company, St. Louis, Missouri; 1 mg/ml at 40° for 4 h) to ascertain the specificity of the labelling pattern. Developed grains were counted at 950 × magnification against a micrometer disc. Fourteen thousand epidermal cells were scanned in each experiment and both the number of labelled cells and the grain density in each were determined. In each case as a control of day to day variations in experimental technique, results were compared to those from the adjacent, clinically normal tissue processed on the same radio-autographic slide. Grain counts from abnormal and normal tissue were tested for statistical differences by the Chi square test.

The response of psoriatic epidermis to 0·1% vitamin A acid ointment, provided by Wallace Laboratories, Cranbury, New Jersey, was evaluated in lesions which had been treated twice daily for 1 week. Control lesions were treated with ointment base alone during this period.

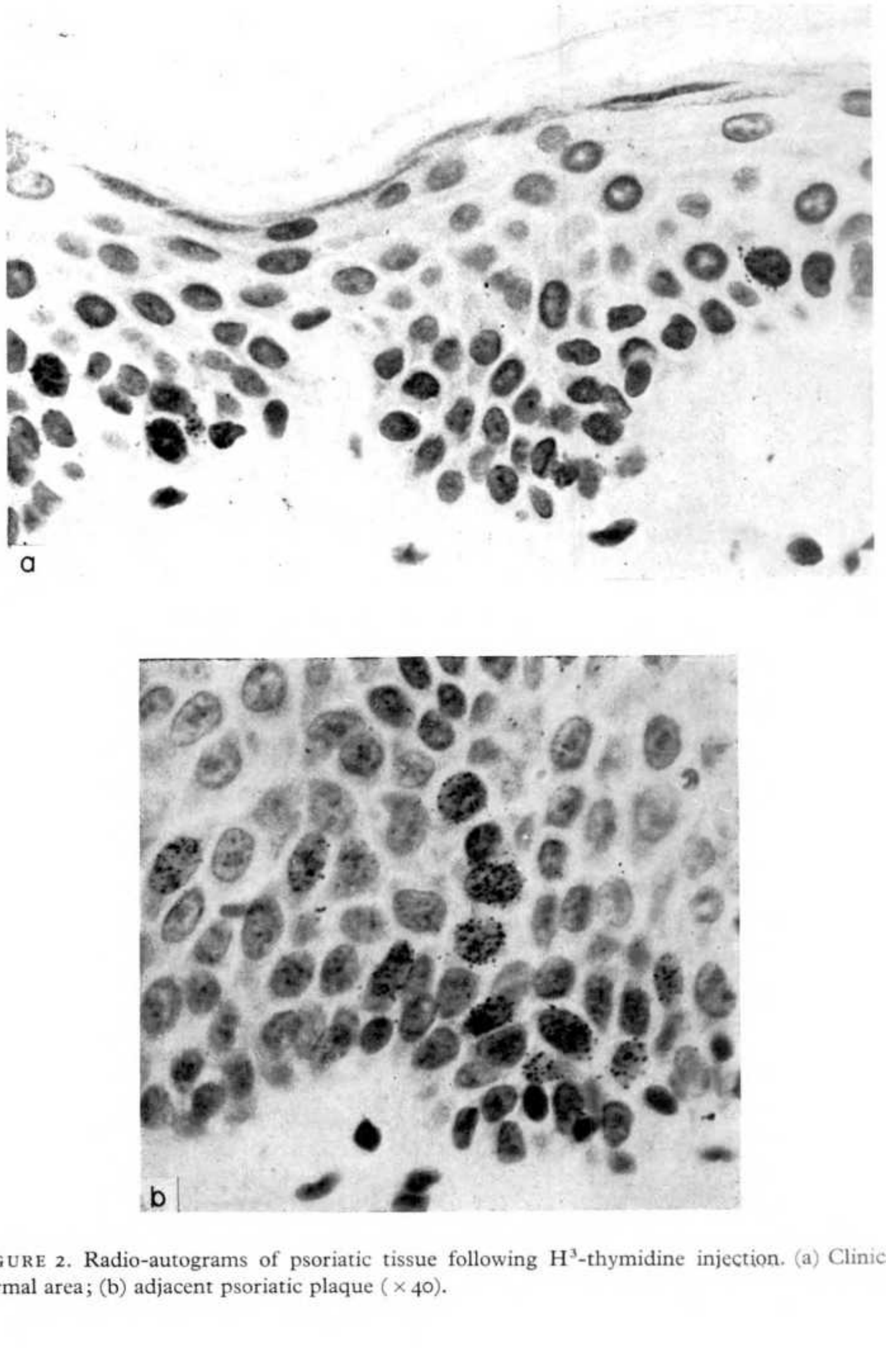

FIGURE 2. Radio-autograms of psoriatic tissue following H^3-thymidine injection. (a) Clinically normal area; (b) adjacent psoriatic plaque (× 40).

RESULTS

Psoriasis

Representative radio-autographic sections from a psoriatic plaque and the clinically normal tissue which surrounds it are displayed in Fig. 2a and b. This tissue was removed 1 h following intradermal injection of tritiated thymidine. The results are summarized in Table 1.

TABLE 1. Summary of radio-autographic data

	Percentage of labelled cells		Grains per cell	
	Normal	Abnormal	Normal	Abnormal
Psoriasis				
Leucine	72·9	62·0*	3·9	3·0
Uridine	61·0	69·6*	2·5	3·0
Thymidine	2·6	6·9*	21·4	16·1
Psoriasis treated with vitamin A acid				
Leucine	89·5	85·5†	6·1	4·3*
Uridine	46·0	76·5*	1·7	3·3*
Thymidine	2·5	4·3*	21·3	19·2
Seborrhoeic keratosis				
Leucine	58·3	67·8*	2·5	2·6
Uridine	60·0	62·1*	3·1	2·9
Thymidine	2·0	1·5*	23·1	26·1
Bowen's disease				
Leucine	87·3	94·7*	4·4	5·6
Uridine	84·8	78·8*	5·2	4·3
Thymidine	1·8	4·0*	30·8	17·2

* Difference between normal and abnormal significant at $P < \cdot 01$.
† Difference between normal and abnormal significant at $P < \cdot 025$.
Other differences are not statistically significant.

A higher percentage of the epidermal cells in the plaque, as compared to the surrounding uninvolved tissue, incorporated thymidine. It can thus be concluded that a larger fraction of the abnormal population was in the S phase of the cell cycle during the time the precursor pool was radioactive. The actual differences may be larger than those shown in the table since intradermal nucleotides rapidly disappear from the injection site by both diffusion and uptake into the vascular tree (Arndt & Freedberg, 1971). Higher blood flow in the psoriatic plaque would also lead to more rapid disappearance of the isotope and thus shorten the effective labelling period. Since these tissues were incubated for relatively short periods to enable practical grain counting, the resultant labelling indices were slightly lower than those found with prolonged incubation periods.

The percentage of cells in the psoriatic plaques which incorporated uridine into RNA was also higher than in control tissue. This difference, although smaller, was also statistically significant. Leucine incorporation, on the other hand, was found in a lower percentage of cells in the plaques than in the surrounding skin. These data were surprising in view of the well documented increases in epidermal protein synthesis associated with psoriasis (Freedberg, 1967), but were readily explained when the data were recalculated to ascertain the number of epithelial cells per unit skin surface area which were involved in protein synthesis. Three times as many leucine labelled cells per cm^2 surface area were found in the plaque as in the surrounding tissue. The differences in nucleic acid synthesis between control and abnormal tissue were also accentuated when the data were analyzed in this way. The average number of grains per active cell was not statistically different in control and abnormal tissue.

In Table 1 are summarized data from twelve psoriatic plaques taken from two patients after 1 week of topical vitamin A acid therapy. Although all analyzed lesions were clinically regressing, a higher percentage of cells in the plaque continued to synthesize DNA, RNA and protein. An alteration was apparent, however, in the synthetic activity of these cells. The magnitude of protein synthesis per active cell as determined by grain counts was decreased when the lesion was compared with similarly treated uninvolved skin. In contrast, the amount of RNA synthesized by each active cell of the plaques was significantly increased. These data indicate a differential effect of local vitamin A acid upon uninvolved and abnormal epithelium. A similar effect is apparent if these post-treatment data are compared to the pre-treatment results. Although direct statistical comparison of these two sets of data is not possible, there is evidence that the fraction of psoriatic cells synthesizing DNA decreases following local vitamin A acid treatment, although at the same time the fraction synthesizing RNA and protein increases. Such differences were not apparent in clinically normal skin from psoriatic patients.

Seborrhoeic keratoses

Representative radio-autographic sections from seborrhoeic keratoses and the surrounding normal skin are displayed in Figs. 3 and 4. The data from these studies are summarized in Table 1. The percentage of cells involved in protein synthesis is significantly greater in the lesions than in the surrounding normal tissue. The small increase in percentage of cells involved in RNA synthesis is also statistically significant. The fraction of cells in the lesions synthesizing DNA was found, on the other hand, to be significantly less than in the surrounding normal skin. No difference was found

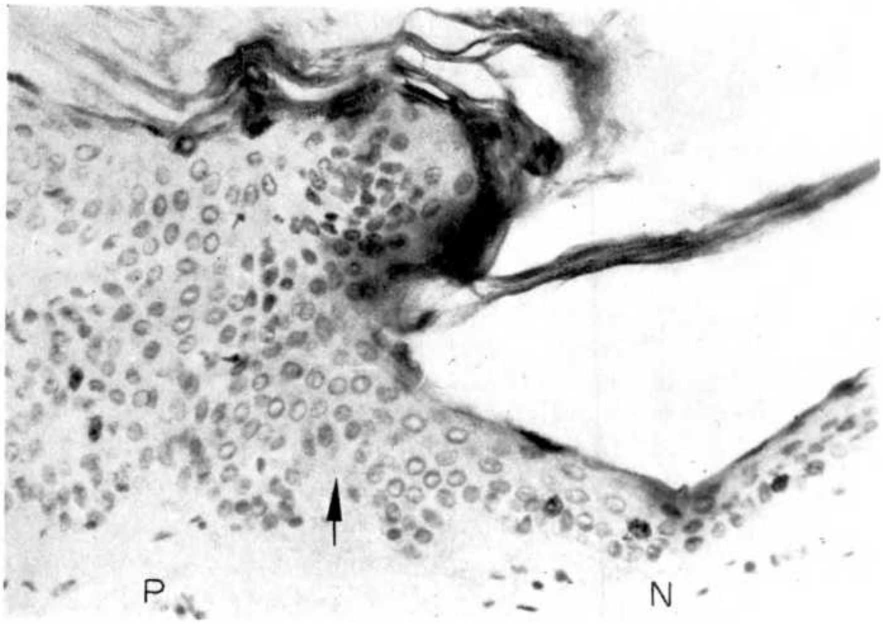

FIGURE 3. Radio-autogram of junction between normal tissue and seborrhoeic keratosis following H^3-thymidine injection. The arrow points to the junction. N, clinically normal; P, pathological tissue (× 40).

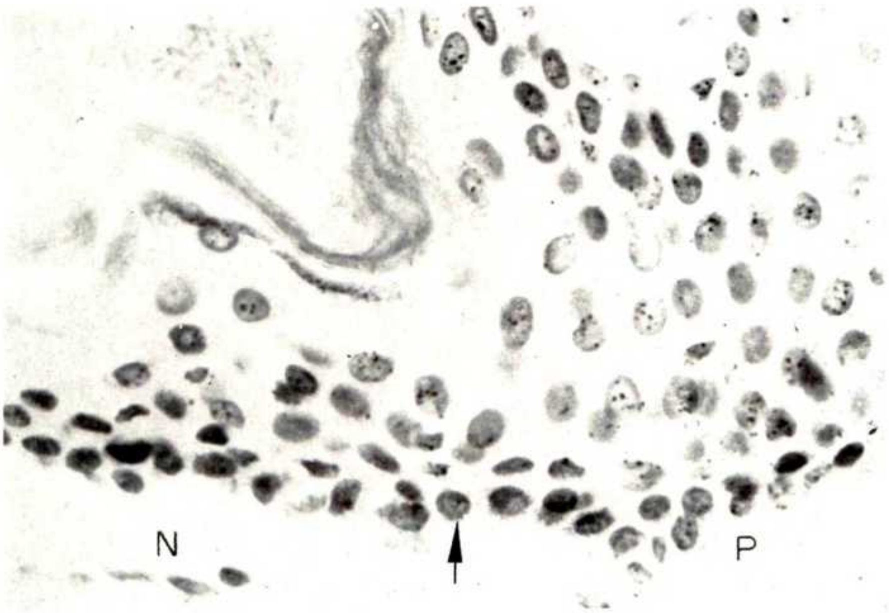

FIGURE 4. Radio-autogram of junction between normal tissue and seborrhoeic keratosis following H^3-uridine injection. Symbols as noted above in Fig. 3 (× 40).

between the synthetic activity of each active cell in the normal and pathological areas.

Bowen's disease

A 27 cm^2 lesion, clinically and histologically consistent with Bowen's disease, was studied following injection of each of the labelled precursors into well-separated areas at the junction of the plaque and the surrounding normal tissue. Representative radio-autograms are reproduced in Fig. 5, and a summary of the data is presented in Table 1. The most obvious abnormality apparent is the two-fold increase in the percentage of DNA synthesizing cells in the lesion. The percentage of cells involved in protein synthesis was increased to a smaller but equally significant extent while the relative size of the RNA synthesizing pool was decreased. Again, the differences in synthetic activity of each active cell, as measured by the number of grains in each labelled cell, were not of statistical significance.

DISCUSSION

This study demonstrates the applicability of currently available radio-autographic techniques to the evaluation of human hyperplastic epidermal disease and its response to topical therapy. The multiple unavoidable variables which would normally affect such studies (for example, variations in synthetic activity in different areas of the body, variability in the injection procedure and in the processing technique) have been effectively eliminated by comparing the pathological tissue to its clinically normal and contiguous control. Caution must be exercised during such studies, however, since the radioactively labelled compounds are rapidly absorbed from the local site of injection (Arndt & Freedberg, 1971). Although the amount of isotope incorporated into epidermal macromolecules results in excellent radio-autograms, the bulk of the injection must therefore be minimized.

The enhanced synthesis of nucleic acids and proteins found in psoriatic epidermis confirms previous

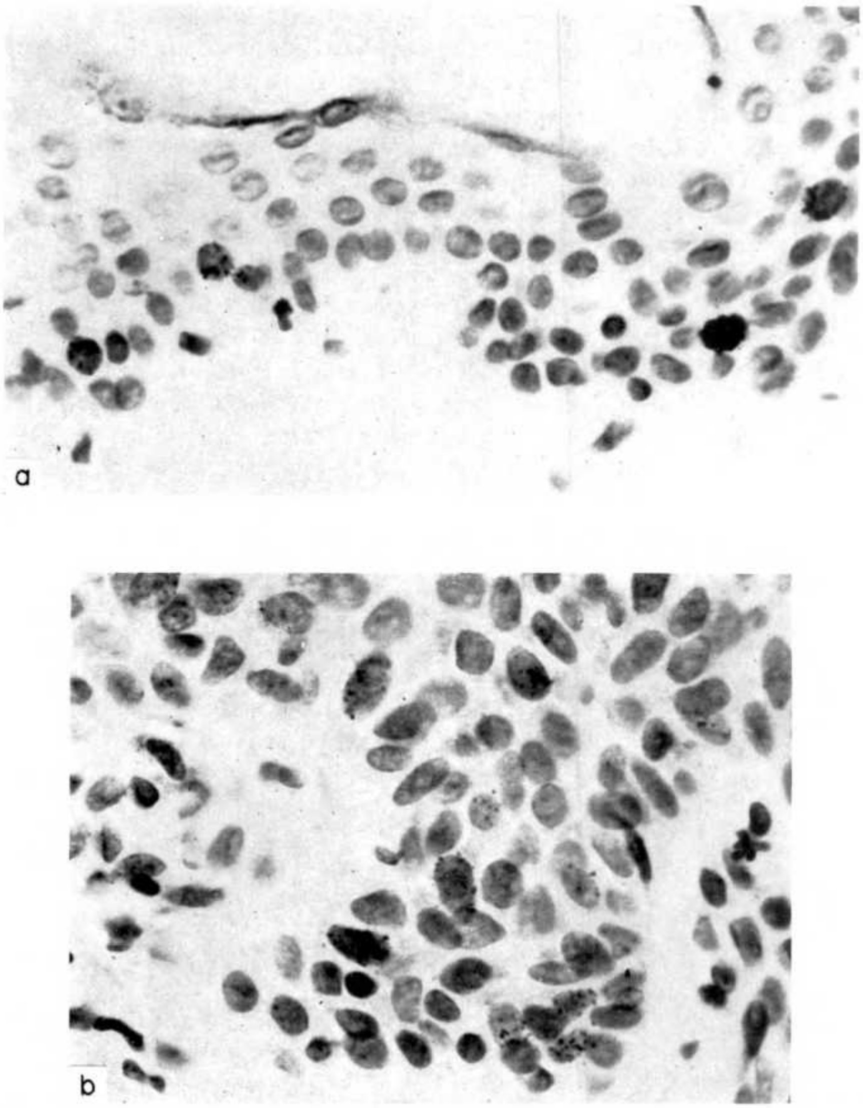

FIGURE 5. Radio-autogram of clinically normal surrounding tissue and lesion of Bowen's disease following H^3-thymidine injection. (a) Normal area; (b) involved tissue (× 40).

data from several laboratories which indicate a generalized increase in macromolecular synthesis in this disease (Van Scott & Ekel, 1963; Freedberg, 1967; Weinstein & Frost, 1969). It can be predicted, therefore, that an effective local medication for psoriasis would be an inhibitor of protein and/or nucleic acid synthesis which shows some specificity for the pathological tissue. Our results indicate that, during the first 2 weeks of therapy, vitamin A acid satisfies these specifications in part. The medication inhibits DNA synthesis in the plaques although incorporation of precursors into RNA and protein is enhanced. We have previously described similar enhancement using other inhibitory compounds in animal model systems (Freedberg, 1965) and believe that stimulation of RNA and protein synthesis is a general phenomenon caused by many irritating substances which subsequently result in inhibition of epidermal proliferation.

Seborrhoeic keratoses, which were included in this study as an example of benign epithelial neoplasia, demonstrate another type of abnormality of macromolecular synthesis. RNA and protein synthesis in the lesion is enhanced at the same time as the percentage of cells synthesizing DNA is decreased. Lachapelle (1969) has published data from seborrhoeic keratoses which demonstrate the same phenomenon. Complete interpretation of the findings, however, depends upon studies of the size of the germinative cell population and its cycle in seborrhoeic keratoses. In addition, data are not yet available concerning the stability of the metabolic pattern throughout the life history of seborrhoeic keratoses. All lesions reported in this communication were of the mature, verrucous variety of chronic duration. Subsequent studies should indicate whether a similar metabolic pattern is found in the more rapidly growing, flat, immature seborrhoeic keratoses.

Bowen's disease was chosen as an example of the next stage of epithelial hyperplasia. Although not overtly malignant, the cells of the lesions deviate even further from normal growth patterns. The enhanced DNA and protein synthesis which we have demonstrated correlates well with the morphological evidence of stimulated macromolecular metabolism obtained by others using both light and electron microscopy (Seiji & Mizuno, 1969). The apparently decreased number of RNA synthesizing cells in the Bowen's lesion may best be attributed to a change in the rate of RNA turnover in this as well as other more malignant processes and indicates another avenue for further study.

ACKNOWLEDGMENTS

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Labelling index in the mucosal lesions of lichen planus

D.M.WALKER AND A.E.DOLBY

Department of Oral Medicine, Dental School, Welsh National School of Medicine, Heath, Cardiff

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SUMMARY

The labelling indices of the epithelium of the lesions of oral lichen planus (LP) have been estimated, using *in vitro* labelling with tritiated thymidine. The relationship between this epithelial labelling index and the underlying inflammatory cell density was also examined. The total labelling activity in the LP lesion was comparable with that of normal mucosa but was redistributed as follows. The labelling index of the basal epithelial layer was increased above that of normal controls and the labelling in the suprabasal layer was decreased. Thus 50% of mitoses occur basally in involved atrophic LP epithelium. The implications of this redistribution of mitotic activity are discussed.

The labelling index of epithelium was reduced where the inflammatory cell density was exceptionally high, but no simple inverse relationship between labelling index and inflammatory cell density was apparent. This supports the concept that the primary event in lichen planus seems to occur in the basal epithelial layer followed by an inflammatory cell response in the underlying connective tissue. Subsequent secondary damage to the epithelium, including inhibition of mitosis, may be produced by this inflammatory infiltrate only if it is very dense.

Lichen planus (LP) is an unexplained inflammatory disorder of the skin and mucous membranes. As it affects the oral mucosa some of its clinical and histological characteristics appear to be contradictory. Thus the epithelium of the papule or stria of oral LP is thickened due to hyperkeratosis or parakeratosis and acanthosis, yet the basal layer of the generative compartment is undergoing liquefaction degeneration. It has been suggested that this degeneration implies a low mitotic rate (Pinkus & Mehregan, 1969).

Conversely, in the atrophic form of oral LP the epithelium is thinned, even ulcerated, yet there is the same liquefaction degeneration of the basal cell layer.

The role of the inflammatory infiltrate in the underlying connective tissue is unknown, although it has been suggested that, in skin LP, damage to the basal epidermal layer is followed by an inflammatory reaction in the sub-epithelial zone (Thyresson & Moberger, 1957; Black & Wilson Jones, 1972). Samman (1972) also suggested that this epithelial degeneration is a primary event.

In this study we have investigated the proliferative activity of the epithelium in the lesions of oral LP and normal mucosa by means of an *in vitro* labelling technique with radioactive isotope. We have also examined the relationship between this labelling index and the inflammatory cell density in the underlying connective tissue.

METHOD

Using local anaesthesia, biopsies were taken of lesions of the buccal mucosa away from the occlusal plane of seventeen patients with acanthotic or atrophic LP. Similar biopsies were taken from ten normal controls with their informed consent. Approximately half of each biopsy was fixed in 10% formol saline and processed for routine diagnostic histology and the remaining portion divided into fragments of not more than 1 mm^2 epithelial surface area. These fragments were then incubated in 1 ml of tissue culture medium 199 (Wellcome Research Laboratories, Beckenham, England) containing 5 μCi of tritiated thymidine (Radiochemical Centre, Amersham, England; specific activity 5 Ci/mmole) on a rotary mixer. After 1 h incubation, the fragments were fixed in 10% formol saline and then routinely processed for sectioning. The sections were then dewaxed, hydrated, dried and coated with an autoradiographic nuclear emulsion (Ilford K5) based on Joftes & Warren (1955). These were stored for 2 weeks in a light-proof container at 4°C and then developed with Kodak D 19 developer and fixed with Kodafix. The labelling index was estimated only in tissue fragments which had been sectioned perpendicularly to the basement membrane zone and in which epithelial cells in the centre of the fragment had more than six grains of silver over their nuclei.

Labelling index

For each subject the total number of labelled and unlabelled basal cells in the sectioned epithelium was counted. The number of labelled suprabasal cells to a height of four cells from the basement membrane was also estimated. The labelling indices were defined as the number of (1) labelled basal, (2) labelled suprabasal, and (3) total labelled cells, per 100 labelled and unlabelled basal epithelial cells. Wherever possible, at least 1000 basal epithelial cells were counted.

Labelling index in relation to inflammatory cell density

To compare the labelling index at any particular part of the epithelium with the density of the inflammatory cells of the corium immediately beneath, the above method was modified as follows.

An eyepiece graticule was used to define an area of epithelium, 0·025 mm square, at right angles to the basement membrane, two epithelial cells in depth. The labelling indices for this area were calculated and then the inflammatory cells included in that square were also counted. This procedure was repeated serially along the epithelium when the configuration of the epithelium–connective tissue interface would permit.

For each patient, the mean labelling index for fields with a mononuclear cell density 0–10, 11–20, 21–30, 31–40, etc., cells per field, was determined.

RESULTS

Labelling activity

The values for labelling indices obtained for normal buccal mucosa are higher than those from other workers, e.g. Toller (1971). We assume that this arises from some difference in our method. However, since this method as described above was rigidly adhered to for both the lichen planus and normal mucosa, the alteration in labelling in LP demonstrated below is not invalidated.

The total labelling indices of epithelium in oral LP lesions did not differ significantly from that of the mucosa of normal subjects (Table 1 and Fig. 1). The labelling index of involved lesional epithelium did not appear to differ from that of adjacent histologically uninvolved epithelium, but sufficient material was not available for this to be confirmed statistically. However, the basal labelling index for the epithelium of LP lesions was significantly ($P<0·01$ and $<0·05$) greater in acanthotic and atrophic LP lesions than in normal control mucosa.

TABLE 1. Mean epithelial labelling indices and mean ratios of suprabasal:basal indices in the mucosal lesions of patients with acanthotic and atrophic lichen planus (LP) and of buccal mucosa of normal controls

	Basal labelling index	Suprabasal labelling index	Ratio of suprabasal : basal labelling indices	Total labelling index
Acanthotic LP 9 patients—2 female, 7 male (Average age 48·3 years)	13·7±1·93*	20·3±3·37	1·73±0·12	33·8±6·00
Atrophic LP 8 patients—4 female, 4 male (Average age 53 years)	7·0±3·84	11·68±2·5	1·06±0·02	23·6±10·0
Normal 10 patients—4 female, 6 male (Average age 37·7 years)	5·62±0·97	34·4±2·72	12·33±2·34	39·9±3·3

* Mean ± SEM, Weinstein & Frost's correction (1969) not applied.

Conversely, the suprabasal labelling indices of acanthotic and of atrophic LP were less than in normal subjects and the differences again were statistically significant ($P<0\cdot01$ and $P<0\cdot001$ respectively). In other words, whereas the total labelling did not differ from that of normal mucosa, its distribution in the lesional epithelium was significantly altered. Therefore, in normal epithelium approximately seven mitoses occurred in the suprabasal layer for every one basal mitosis, but in acanthotic LP the ratio had become reduced to 1·5 : 1 and in atrophic LP equal numbers of mitoses occurred in suprabasal and basal layers (Fig. 2 and Table 1).

Labelling index of the epithelium in relation to the inflammatory cell density in the connective tissue

Our results did not demonstrate conclusively a direct relationship between the labelling index at any

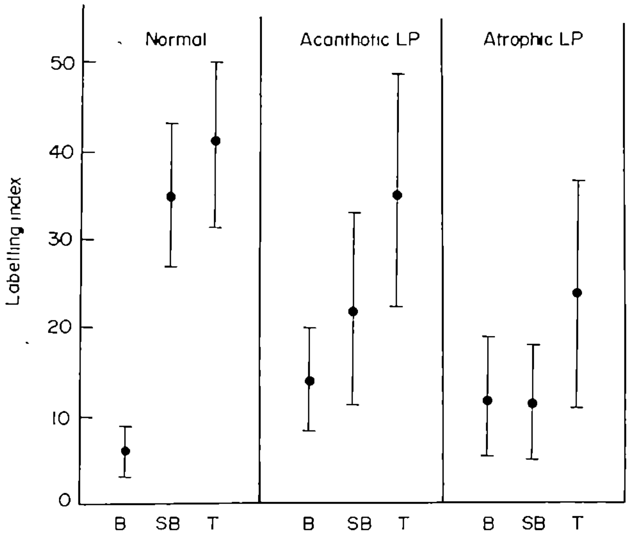

FIGURE 1. Labelling index in epithelium of normal buccal mucosa and oral lichen planus (LP) (acanthotic LP and atrophic LP). B, basal; SB, suprabasal; T, total.

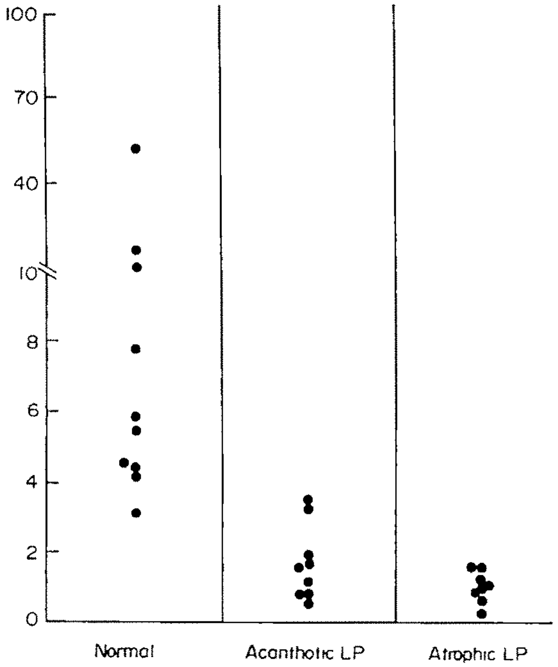

FIGURE 2. Distribution of mitotic activity in lichen planus. Ratio of number of suprabasal : basal mitoses in lichen planus (LP) compared with normal.

defined point in the epithelium in LP and the local inflammatory cell density in the connective tissue immediately beneath. However, labelling indices for epithelium overlying the corium, where the inflammatory cell density was exceptionally high, did appear to be reduced (Fig. 3).

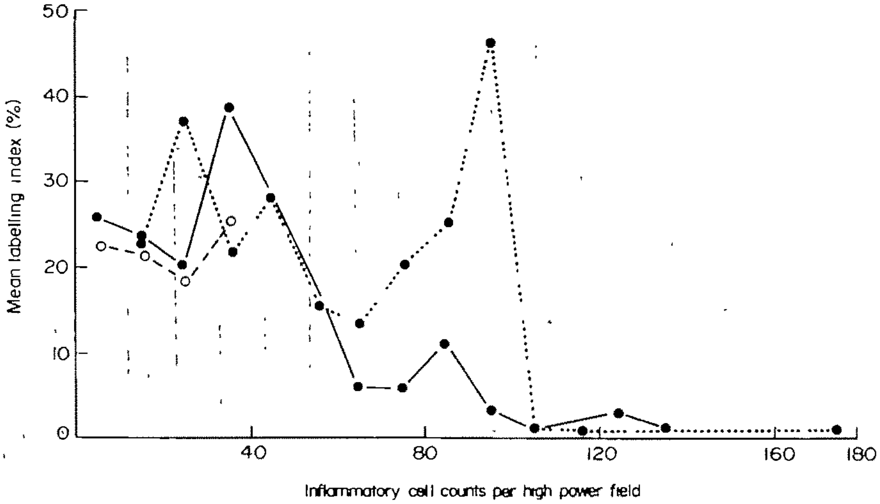

FIGURE 3. Labelling index in the mucosal lesions of lichen planus. - - -, normal; ——, acanthotic lichen planus; . . . . ., atrophic lichen planus.

DISCUSSION

Allowing for the fact that the patient group was on the average older, the total labelling activity of involved epithelium in acanthotic and atrophic LP did not differ significantly from mucosa of normal subjects. We have not found, therefore, that changes in epithelial thickness in LP lesions are due simply to an increase in mitotic activity. This agrees with the work of Marks, Black & Wilson Jones (1973), who found that the involved epithelium of cutaneous lesions of LP had a slightly lower labelling index than that of adjacent uninvolved skin. Their kinetic studies suggested that repopulation of the involved lesional epithelium was occurring by migration from proliferating epithelium at the margins of the lesion. We did not observe the increased labelling at the periphery of our mucosal lesions found in their study on the skin, neither were the spindle-shaped labelled keratinocytes reported by these workers identified. As Marks *et al.* (1973) emphasized, caution must be observed in interpreting labelling indices where there is severe erosion of the basal layer of the LP lesion.

An alternative mechanism to explain the change in thickness of LP epithelium without any increase in mitotic rate has been put forward by Black & Wilson Jones (1972), who pointed out that the epidermal cells in active areas were larger than those in the unaffected skin. Again, Christophers & Petzold (1969) have shown experimentally that two different areas of epidermis may have identical labelling indices, yet vary in thickness. They showed that the thicker epidermis had an increased epidermal

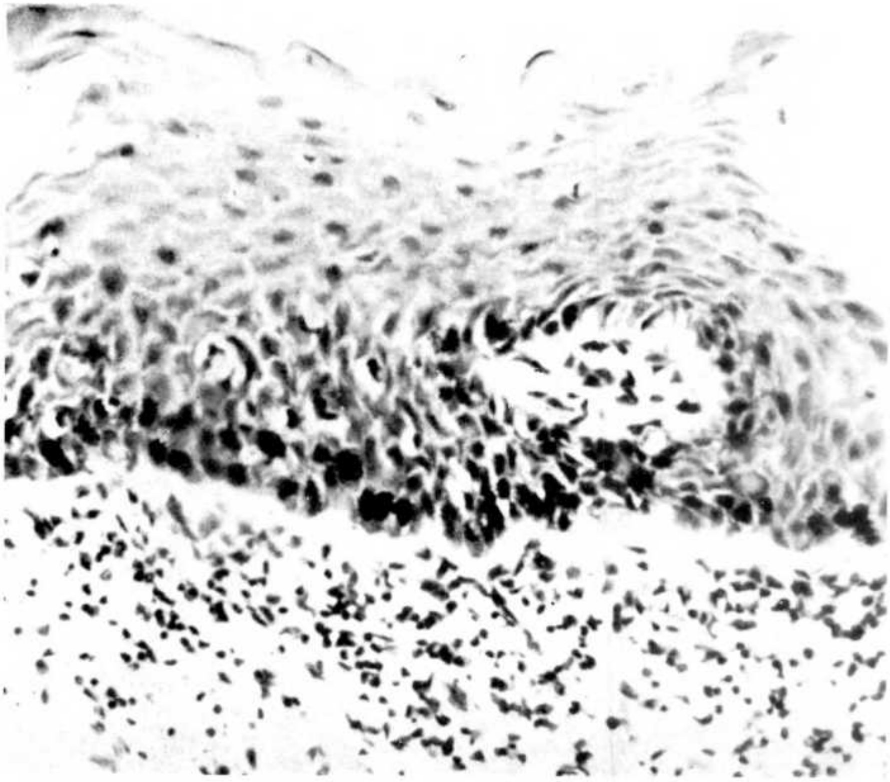

FIGURE 4. Radio-autograph of epithelium of LP lesion of buccal mucosa with only moderate density of inflammatory infiltrate. Labelling index comparable with that of normal mucosa in Fig. 5 (Haematoxylin, × 63).

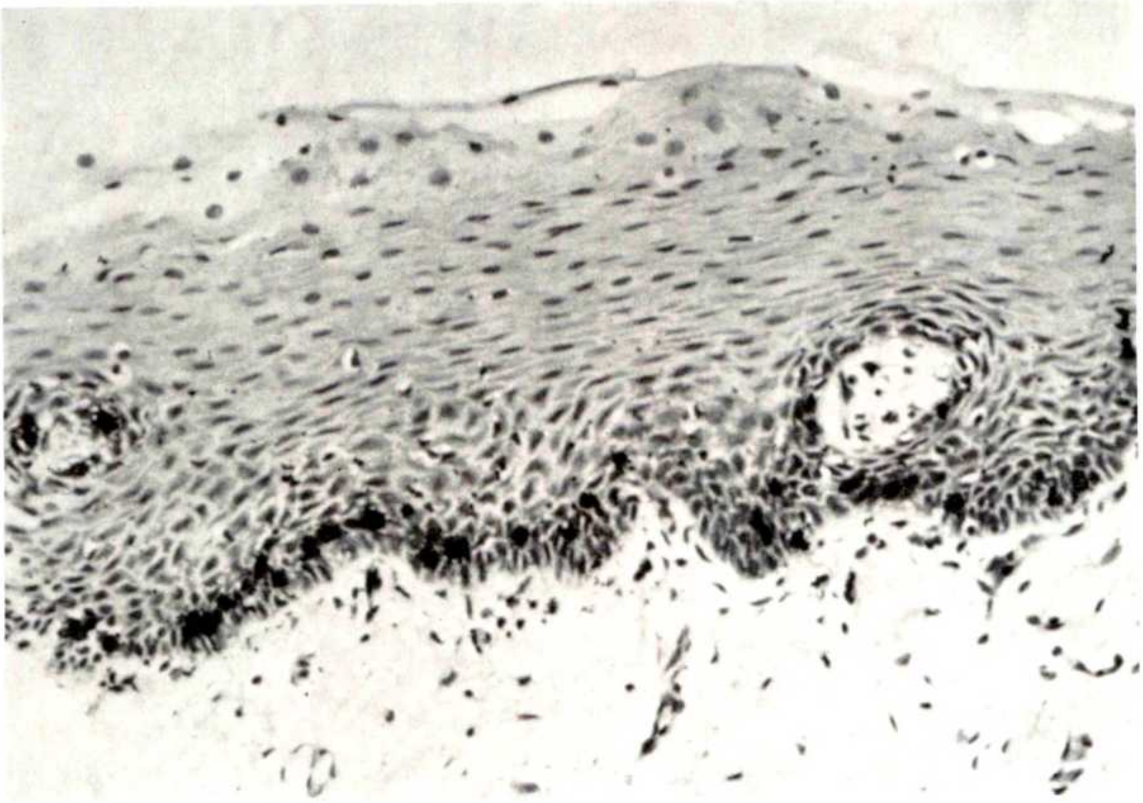

FIGURE 5. Radio-autograph of section of normal buccal mucosa (Haematoxylin, × 63).

transit time. This slowing of the epithelial transit time may account for the thickening of the epithelium in the lichen planus lesion.

The total labelling indices in atrophic LP were lower than in acanthotic LP, but the difference was not significant statistically. By contrast, El-Labban, Lucas & Kramer (1971) found acanthotic LP to have lower mitotic values than atrophic LP, but again the difference was not significant. However, their method relied on identifying mitotic figures by light microscopy which, it is suggested, almost invariably identifies fewer mitoses (Lachapelle & Gillman, 1969).

The significant increase in basal labelling index and decrease in suprabasal labelling index have not been reported before. Although this apparently altered distribution of mitotic activity in the LP lesion (reflected by reduction in the suprabasal to basal ratio) is interesting, too much emphasis should not be placed on it in view of Loe, Karring & Hara's (1972) evidence that almost all mitoses, which in individual sections may appear to be superficial to the deepest two cell layers, are in fact situated next to the basement membrane. One interpretation of this apparent redistribution of mitotic activity in LP is that, since the epithelium-connective tissue interface usually becomes flattened in the oral lesion, mitoses which have appeared suprabasal in the convoluted normal epithelium, are revealed to be actually basal.

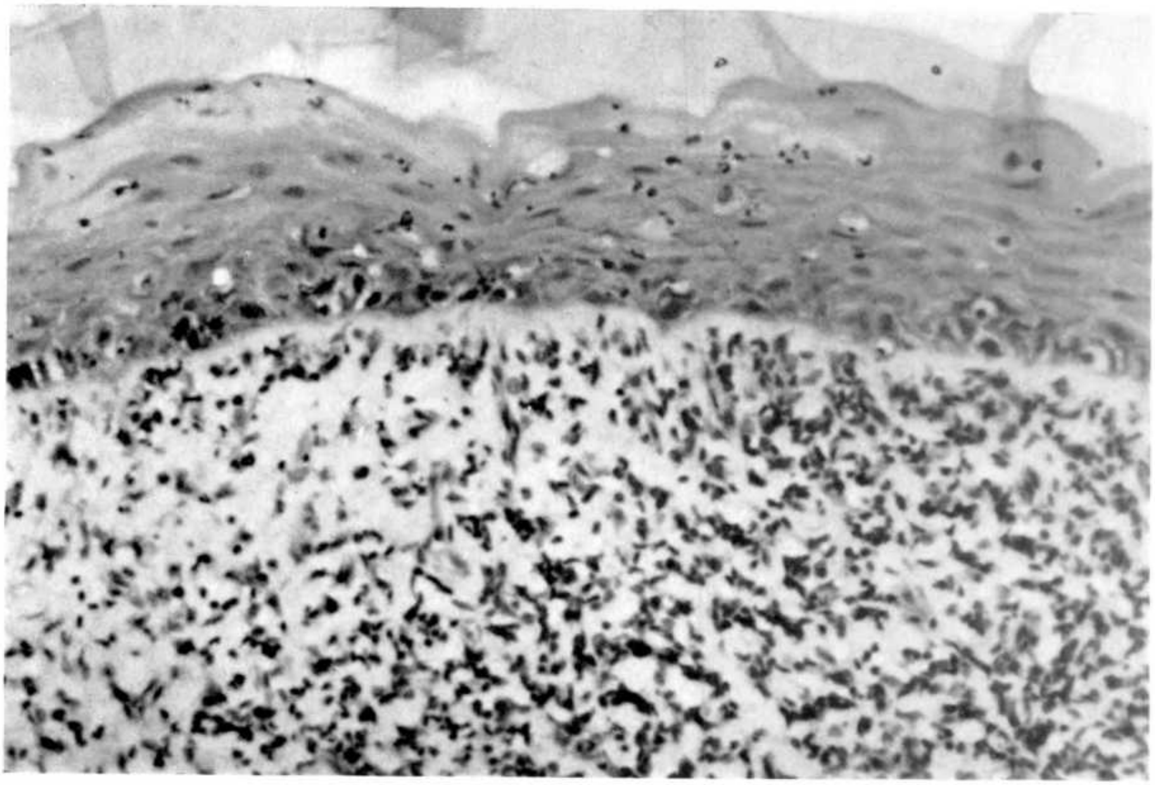

FIGURE 6. Radio-autograph of atrophic LP lesion of buccal mucosa of same patient as Fig. 5. At this high inflammatory cell density, the epithelial labelling is reduced (Haematoxylin, × 63).

Labelling index in relation to inflammatory cell count

Although the labelling index was lowest in those areas of epithelium where the connective tissue inflammatory cell infiltrate was most dense, these two variables did not show a simple inverse relationship (Fig. 3). Thus at a moderate inflammatory cell density the epithelium of the LP lesion in Fig. 4 has mitotic activity comparable with that of the normal control (Fig. 5); this falls off only at higher inflammatory cell density (Fig. 6); that is, no mathematically proportional inhibition of labelling activity in the epithelium by the inflammatory infiltrate could be demonstrated. This is in agreement with our previous finding in oral LP that the patient's serum or lymphocytes are not more cytotoxic for oral epithelial cells compared with serum or lymphocytes from normal controls (Dolby & Slade, 1973). Lachapelle & de la Brassine (1973) identified this LP inflammatory infiltrate as consisting of lymphoblasts, again suggesting that the primary event in the pathogenesis of LP may be an antigenic change in the basal layer of the epithelium which subsequently stimulates an immune cellular response in the dermis. Our tentative suggestion is that secondary changes in the epithelium, including inhibition of mitotic labelling, may occur as a subsequent reaction to the presence of these inflammatory cells or their soluble products only when their concentration reaches a sufficiently high level.

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Carriage of *Corynebacterium acnes* in school children in relation to age and race

MUNA MATTA*

Department of Bacteriology, St John's Hospital for Diseases of the Skin, London E9 6BX

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SUMMARY

The prevalence of *Corynebacterium acnes* on the foreheads of male and female children of caucasoid and negroid origin has been investigated using a semi-quantitative technique. Few organisms were recovered from children of less than 12 years of age. The increase in count occurred earliest in negroid girls and latest in caucasoid boys. This may be related to the age of puberty.

The incidence of acne vulgaris among different races has been reported by several investigators (Fox, 1930; Ziemann, 1930; Goodman & Burr, 1934; Hazen, 1935; Hinrichsen, 1938; Hamilton, Terada & Mestler, 1964; Wilkins & Vorrhaes, 1970). These reports were all based on clinical observations, however, and no bacteriological studies appear to have been done. Burton *et al.* (1971) have reported on the prevalence of clinical acne in adolescence; but only in caucasoid children. This paper reports the relative prevalence of *Corynebacterium acnes* in school children aged from 5 to 17 years of caucasoid and negroid origin

MATERIALS AND METHODS

A total of 1100 children was included in this study; the age range was 5 to 17 years; 622 were of caucasoid origin and 478 were negroids; 504 were male and 596 were female (see Table 1). Males and females were included irrespective of the presence of clinically apparent acne. The work reported here was carried out during visits to schools in the London Borough of Hackney; according to the figures published by the Registrar General, about one-fifth of the inhabitants were born outside the U.K. and about half of these are of negroid origin. Most of the children have been born in the borough, however. The various groups are well integrated, indigenous and immigrant groups living in the same streets and attending the same schools. There is therefore comparability, if not equality, in the social status of the various groups. This study was carried out at the same time as a survey on the carriage of pyogenic cocci in the population (Noble, 1974).

The survey was carried out by attending the school accompanying the school nurse, often, but not always, at a routine school hygiene inspection. Letters of consent for swabbing were sent to the parents before the visit. It was possible to examine only those agreeing to the survey, but from other evidence (Noble, 1974) it seems unlikely that there was any particular bias between those agreeing to the survey and those refusing permission. Age, sex and apparent race were recorded at the time of the school visit; children of Indian or Chinese origin and those of known mixed racial origin were omitted from the analysis. Those receiving antibiotics were also omitted.

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* Present address: Department of Dermatology, American University Hospital, Beirut, Lebanon.

The face and back were inspected at thesame time as the swabbing was done and any grade of acne or treatment for acne was recorded. Grading of lesions was based on that of Burton *et al.* (1971).

Using moist calcium alginate swabs, samples were taken from the forehead by swabbing an area of about 10 cm^2 for about 10 s. The swabs were then placed directly into phosphate buffer pH 7·0 and shaken vigorously on a mechanical agitator to dissolve and disperse the alginate; ten-fold dilutions were made without delay. These suspensions were inoculated onto Brewer's anaerobic agar (Difco) without dextrose or indicator. The petri dishes were incubated anaerobically in a MacIntosh & Fildes jar for 7 days at 37°C. Colonies of presumptive *C. acnes* were counted and representatives re-inoculated onto blood agar (Oxoid blood agar base containing 5% horse blood) and incubated aerobically to test oxygen requirements. Only obligate anaerobic diphtheroids are considered in this study and are reported as '*C. acnes*'. Attempts were made to distinguish between type I and type II *C. acnes* on morphological grounds (Voss, 1970).

RESULTS

Table 1 shows the range of total counts of *C. acnes* isolated from the foreheads; this is expressed as mean count per cm^2 per person. Table 1 also shows the distribution by age, sex and race of the children sampled. In the younger age groups there was little variation in the count but the older groups showed wide variation with the gradual shift to larger numbers. Fig. 1 gives the logarithmic mean count of *C. acnes* per cm^2 in each age group. There was a gradual rise in the count with increase in age; females of negroid origin showed an earlier rise than any other group; female subjects in each

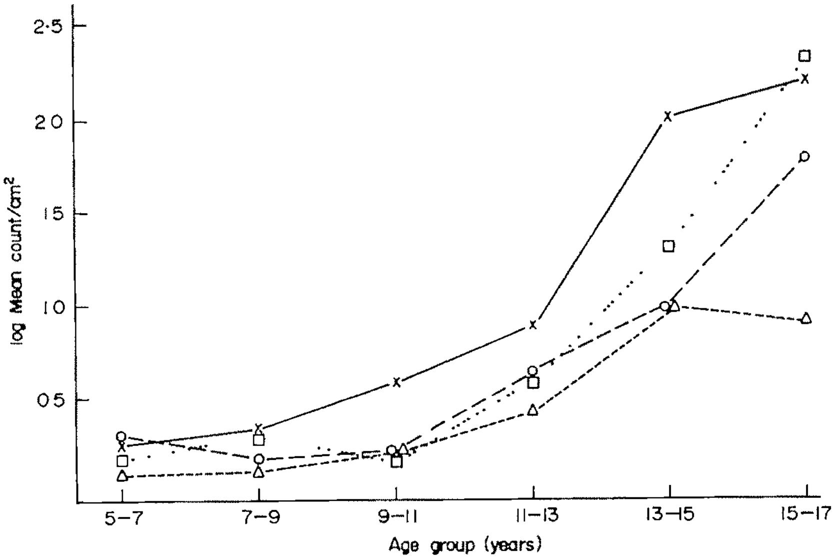

FIGURE 1. Log. count of '*C. acnes*' per cm^2 of forehead in relation to age, sex and race. ×——×, female negroid; □.....□, male negroid; ○———○, female caucasoid; △---△, male caucasoid.

TABLE 1. *C. acnes* expressed as total count per cm² in male (M) and female (F) school children, in the caucasoid (C) and negroid (N) races

Age (years)	Total	Sex	0		1–10		10–50		50–100		100–300		300–500		500–700		700–1000		1000–5000		5000–10,000		10 000–20,000		20,000–100,000	
			C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N
5	48	M	17	20	2	9	—	—																		
	48	F	13	15	7	11	2	—																		
6	55	M	18	20	7	10	—	—																		
	51	F	13	16	10	9	—	3																		
7	57	M	19	15	9	11	1	2																		
	46	F	21	11	8	5	1	—																		
8	39	M	16	19	—	3	—	1																		
	49	F	14	16	3	10	1	5																		
9	56	M	23	22	7	4	—	—			—	—														
	59	F	25	19	5	5	—	4			—	1														
10	68	M	24	19	11	9	3	2	—	—	—	—	—	—	—	—										
	67	F	21	9	12	13	1	7	—	1	—	1	—	1	1	—										
11	54	M	14	8	13	10	4	4	—	1			—	—												
	53	F	17	8	11	5	5	3	—	3			1	—												
12	27	M	10	1	7	6	3	—			—	—			—	—			—	—			—	—	—	—
	38	F	11	1	10	7	1	1			1	2			—	1			1	—			1	—	1	—
13	16	M	1	1	10	1	1	—	—	—	—	—			—	—	—	—	1	—	1	—	—	—		
	46	F	4	—	16	2	9	1	1	1	2	3			—	1	—	1	—	2	—	1	—	1		
14	53	M	7	4	19	5	2	4	1	—	6	1	—	—	—	—	—	—	1	1	—	1	—	1	—	—
	112	F	24	4	32	10	10	3	2	1	3	5	1	—	1	—	1	—	2	2	1	—	2	4	2	2
15	16	M	3	—	5	2	3	—	—	—			—	—	—	—			—	1	—	—	—	2		
	17	F	1	2	5	1	—	1	—	1			1	—	—	1			—	—	1	1	—	2		
16	15	M	2	—	5	1	1	2			1	—	1	—	—	—	1	—	—	—			—	1	—	—
	10	F	—	2	1	—	—	—			—	1	—	1	—	1	—	—	1	—			1	1	—	1

TABLE 2. Subjects with *C. acnes* count higher than 500 cells/cm^2

	No acne		Acne	
	Caucasoid	Negroid	Caucasoid	Negroid
Males	0	2	3	5
Females	2	12	13	4

race showed an earlier rise than the corresponding males, except in the 15–17 years age group where there is a slight overlap in the negroid subjects. Finally, Fig. 1 shows a higher average count in the negroid race than in the caucasoids.

Table 2 shows the subjects who had counts greater than 500/cm^2; 31% of subjects with high counts had no clinical evidence of acne; 88% of these individuals were of negroid origin, the majority being female. On the other hand, 62% of the sixty-five subjects with clinical acne had counts lower than 500/cm^2. The distribution of clinically apparent acne in relation to age and race is shown in Table 3.

A series of eighty-five children, aged 11–14, contributed to a study of the relative frequency of the two types of *C. acnes*. Type I was the predominant type in fifty-four and type II in thirty-one (type was based on colony morphology only). More caucasoids than negroids carried type II, but the difference was not statistically significant.

TABLE 3. Clinically apparent acne* in relation to age and race

	Caucasoid				Negroid			
	Female		Male		Female		Male	
Age (years)	Total in group	Affected (%)	Total in group	Affected (%)	Total in group	Affected (%)	Total in group	Affected (%)
10–11	69	12	67	3	51	12	53	2
12–13	59	14	34	12	25	12	9	0
14+	91	15	58	19	46	9	26	15

* Only one other child, a caucasoid child aged 9, was found to have clinically significant lesions. In assessing lesions a modified version of the Burton *et al.* (1971) scheme was used. 'Clinically apparent acne' thus indicates lesions consisting of a few comedones and a few papules or pustules.

DISCUSSION

The microbiology of acne vulgaris lesions has been investigated by many and is conveniently summarized by Marples, McGinley & Mills (1973). In the study reported here the emphasis has been on the obligate anaerobic diphtheroids alone, since these have been reported as the most significant organisms in acne lesions.

The technique of sampling used in this study has its shortcomings but it proved to be a simple one for screening large numbers of children. The technically more satisfactory, quantitative technique used by Williamson (1965) would have been impossible to carry out in these surveys. The findings represent the relative incidence of *C. acnes* on the surface of the skin amongst different individuals, and in those with no clinical evidence of acne are a fair reflection of the flora of the pilosebaceous units. In subjects with clinical evidence of acne, the method is of limited value if one assumes that some or many of the pilosebaceous units are blocked and do not allow *C. acnes* free access to the surface. This may explain some of the low counts in subjects with acne vulgaris in this study.

The overall findings are parallel to those of other investigators. The rise in the count of *C. acnes* at about the onset of puberty accords well with the findings of Burton *et al.* (1971) on the prevalence of acne in adolescence. The earlier rise in count among females of negroid origin may be a reflection of the earlier pubertal age of the females of this race, though precise data on pubertal age in this population is lacking. Similarly, the females of both races show an earlier rise in count than do their male counterparts. There is a suggestion that negroid individuals of both sexes carry more *C. acnes* than do caucasoids, though this may merely reflect a lack of maturity in the 15–17 year age group studied. Too few cases of acne vulgaris of Grade 2 were seen to permit adequate analysis of the results, though these were in general accord with those of Burton *et al.* (1971).

The incidence of acne vulgaris in different races has been reported previously. Goodman & Burr (1934) found acne vulgaris to be much higher in Jews than in Gentiles, and Hamilton *et al.* (1964) found that there was a greater tendency to acne among white Americans than among Japanese. Comparisons of the incidence of acne among negroids and caucasoids have been made more frequently; the results are variable and conflicting (Fox, 1930; Ziemann, 1930; Hazen, 1935; Wilkins & Vorrhaes, 1970), but this may be related to the difficulty of assessing mild acne on deeply pigmented skin.

ACKNOWLEDGMENTS

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A case of purpura hyperglobulinaemica of Waldenström studied by skin immunofluorescence

W.H.PERKS, F.GREEN AND M.H.GLEESON*

Crumpsall Hospital, Manchester M8 6RB, and
The Department of Pathology, University of Manchester

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SUMMARY

A case of purpura hyperglobulinaemica of Waldenström is reported. Immunofluorescence of skin and subcutaneous tissue using monospecific fluorescein-tagged anti-human IgA, IgG, IgM and complement showed deposition of IgA in the walls of intradermal vessel in affected skin. It is suggested that these findings help in the elucidation of the pathogenesis of the purpura.

First described by Waldenström in 1943, purpura hyperglobulinaemica is a syndrome of recurrent purpura and elevated serum globulins. Typically the patient presents with a petechial rash, which may be slightly elevated or erythematous and may become confluent. Its distribution is predominantly on the lower extremities, less commonly on the arms and trunk, and may be heralded by a sensation of coldness and tingling. The lesions become brown and fade over the course of several days, occasionally leaving a uniform brown discolouration. Further outbreaks of purpura may occur at intervals of days, months or years (Strauss, 1959).

The rise in serum globulins is due to an increase in the gamma fraction. The serum albumin concentration is normal or low and the ESR is invariably raised. The patient may or may not be anaemic (Carr & Heisel, 1966); the bone marrow may show an increase in reticuloendothelial cells and plasma cells or, more commonly, no abnormality. The finding of a positive rheumatoid factor in hyperglobulinaemic purpura is as common as in rheumatoid arthritis (Waldenström & Winblad, 1958). Serum immunoglobulin quantification usually shows raised IgG levels and lesser elevations of IgA and IgM (Carr & Heisel, 1966).

The histological changes in the skin in the early stages after an acute attack consist of a vasculitis with perivascular cuffing by acute inflammatory cells, focal extravasation of red blood cells and focal arteriolar necrosis. After several days the perivascular cuffs are composed of mononuclear inflammatory cells and there is iron, both in the macrophages and free in the dermis (Hambrick, 1958).

CASE REPORT

The patient was a Caucasian male aged 45. In March 1961 he was admitted to Stockton-on-Tees

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* Reprint requests to: Dr M. H. Gleeson, M.6 Unit, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL.

Hospital with symptoms of long-standing duodenal ulceration. A Polya gastrectomy was performed. Two months post-operatively he was admitted to Manchester Royal Infirmary because of intestinal obstruction due to adhesions. During convalescence he developed a purpuric rash.

In June 1962 he was re-admitted to the same hospital complaining of vomiting. Examination at this time showed a purpuric rash which was distributed mainly on the lower extremities, and an arthropathy affecting the hands and wrists. At this time, investigations showed a hypochromic microcytic anaemia with a normal platelet and white cell count. The serum thymol turbidity was 4·4 units (normal range up to 4 units). Electrophoresis of serum proteins showed a normal serum albumin concentration and an increased serum globulin concentration.

Over the next 6 years he was regularly re-assessed and despite therapy with corticosteroids there was no improvement in the rash. During follow-up the serum globulins were persistently elevated (1968, 4·0 g/100 ml; 1969, 5·1 g/100 ml; and 1972, 4·8 g/100 ml).

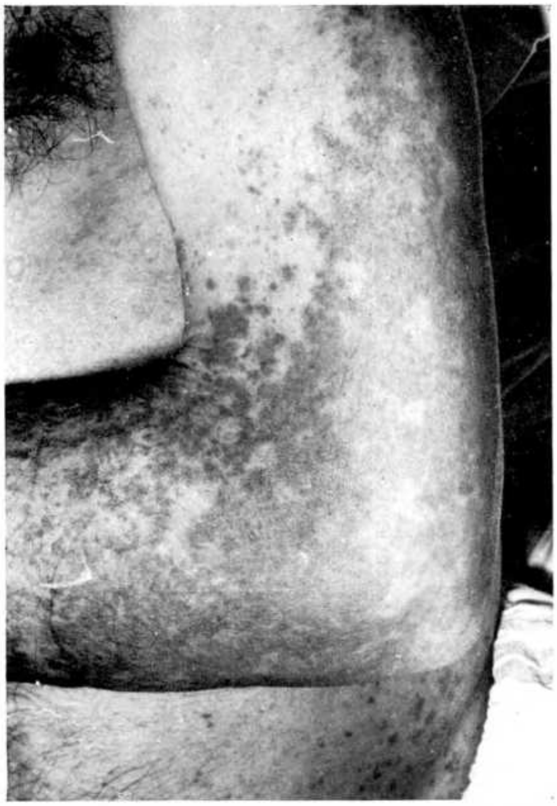

FIGURE 1. Characteristic rash on elbow (late stages).

Between 1968 and 1972 the patient was re-admitted to hospital on four occasions with symptoms suggestive of pulmonary embolism—haemoptysis, pleuritic chest pain and dyspnoea. On admission to Crumpsall Hospital in February 1972 the purpuric rash was again noted (Fig. 1). The patient was dyspnoeic. There was hepatomegaly and marked superficial thrombophlebitis in both lower limbs.

Laboratory data

Investigations in 1972 showed a haemoglobin of 12·1 g/100 ml with a packed cell volume of 39%

and a mean corpuscular volume of 99 μm^3. Platelet count, absolute and differential white cell count and coagulation studies were normal. Other normal investigations included blood urea and electrolytes, serum calcium, phosphate and alkaline phosphatase. serum folate and B_{12}. The serum iron was low at 20 μg/100 ml but the iron binding capacity was normal (360 μg/100 ml). The serum thymol turbidity was 3 units. Cryoglobulins were not detected. There was a markedly raised serum lactate dehydrogenase of 1560 iu/ml. Urine examination was normal and in particular there was no detectable Bence–Jones protein. Studies for malabsorption were negative. A Latex screening test for lupus erythematosus was negative and no LE cells were seen. There was a positive Latex test for rheumatoid factor. The sternal marrow was normal. Electrocardiographs, chest X-ray and lung scan all showed features consistent with pulmonary emboli.

The serum immunoglobulins were as follows: IgA, 800 mg/100 ml (normal 125–425); IgG, 2000 mg/100 ml (normal 600–1600); and IgM, 84 mg/100 ml (normal 50–170). The serum C3 complement level was normal and the direct Coombs' test was negative. Routine serological examinations for syphilis were negative.

Histology and immunofluorescence studies

Skin biopsies taken during the last hospital admission were snap frozen in isopentane, cooled by solid carbon dioxide, and serial sections were cut in an open top cryostat at a thickness of 5 μm. Specific

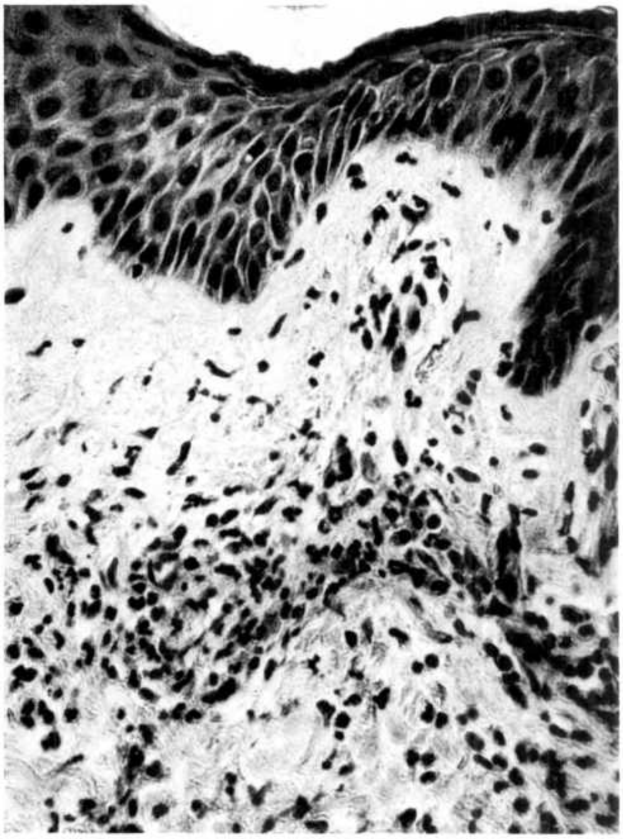

FIGURE 2. Dermal vasculitis (H & E, × 90).

freeze dried anti-sera prepared in swine against human IgA, IgG, IgM and complement, conjugated with fluorescein isothiocyanate and rendered monospecific, were used as in the technique described by Green & Fox (1972). A biopsy of skin from an involved area of the left thigh was compared with uninvolved skin from a similar site. In the latter, histology and immunofluorescence confirmed that it was normal skin. The dermal blood vessels of affected skin showed a vasculitis with perivascular infiltration by polymorphs and eosinophils and extravasation of red cells and leukocytoclasis (Fig. 2). There was marked fluorescence in the affected blood vessel walls and adjacent connective tissue after treatment with fluorescein-tagged anti-human IgA (Fig. 3). There was no comparable fluorescence with fluorescein-tagged anti-human IgG, IgM nor β_1C complement.

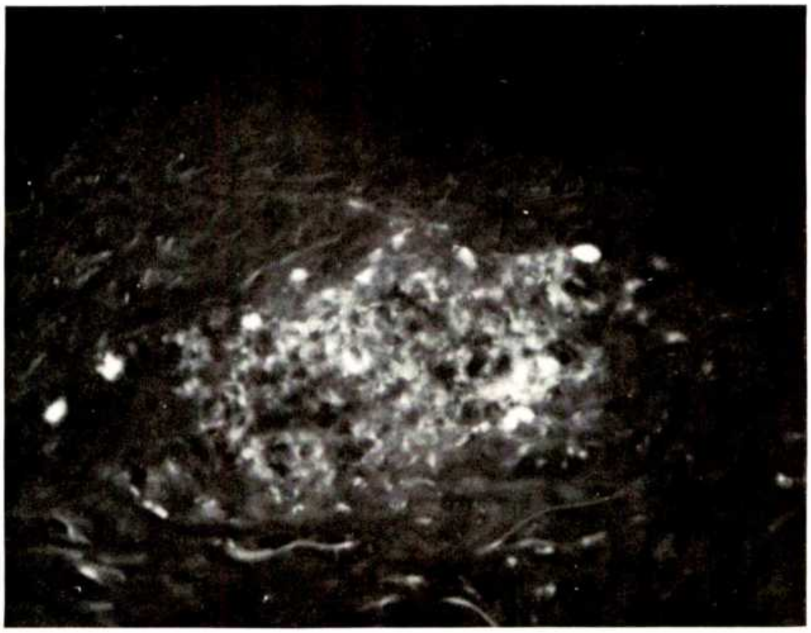

FIGURE 3. Section of dermis stained with fluorescein conjugated anti-human IgA showing diffuse vascular and perivascular fluorescence (× 90).

Course and progress

The patient was initially treated with heparin infusion, oral anticoagulants and oral iron with rapid improvement in symptoms. He has remained well on subsequent follow-up although the rash has persisted.

DISCUSSION

The clinical findings in this patient, namely purpura, hyperglobulinaemia, anaemia and positive rheumatoid factor with raised levels of IgA and IgG, are similar to those reported in other patients with this syndrome.

The frequent association of this syndrome with Sjögren's syndrome, systemic lupus erythematosus and polyarthritis (Hambrick, 1958), together with the finding of a positive rheumatoid factor and an immunoglobulin abnormality, led Waldenström (1968) to consider it to be one of the prototype polyclonal hypergammaglobulinaemias and to suggest an auto-immune basis for the disease. Capra, Winchester & Kunkel (1971) have shown that antiglobulins are present in the sera of these patients. These antiglobulins are usually, but not always, of significantly restricted heterogeneity, having many of the properties of myeloma M proteins. The monoclonal nature of these antiglobulins is difficult to detect on routine cellulose acetate electrophoresis in view of the fact that they are usually complexed with polyclonal γ globulin.

Possibly of relevance to the pathogenesis of the vasculitis is the finding of intermediate immune complexes, composed of γ globulin and anti-γ globulin sedimenting between 7 and 19 Svedberg units, in the sera of most patients with hypergammaglobulinaemic purpura; these complexes are considered to be an essential feature of this syndrome. (Capra *et al.*, 1971). These authors found that the antibody component of these complexes is usually of the IgG class, though IgA antiglobulins have been demonstrated in this disease (Abraham *et al.*, 1970).

The present study is the first to demonstrate the presence of immunoglobulin deposits in the walls of vessels affected by the vasculitis. Carr & Heisel (1966), studying three patients with this condition, demonstrated mild to moderate immunofluorescence in epidermal cells, but no fixed antibody in or around blood vessel walls. Kyle *et al.* (1971), in seven cases studied by skin immunofluorescence, did not demonstrate immunoglobulins in the vessel walls though they did show IgG in dermal mononuclear cells in three cases and β1C in the vessel wall of one case. These apparently cnoflicting findings could be accounted for by the rapid clearance of immune complexes from the lesions. Cream, Bryceson & Ryder (1971) have shown that antibody and complement are cleared within 28 h from guinea-pig skin Arthus reactions. Certainly the clinical manifestations of the skin lesions in this condition are constantly changing and thus the timing of immunofluorescence studies of the skin may be a critical factor.

The finding in the present study of IgA deposits only in the walls of vessels affected by the vasculitis, when taken in conjunction with the high circulating levels of IgA, does suggest that the vasculitis is immunologically mediated. The mechanism involved is less obvious though it is very possibly initiated through the formation of immune complexes (Capra *et al.*, 1971). The presence in this case of a normal serum complement together with the absence of complement in the lesions make it unlikely that the vasculitis is mediated by the complement system. Furthermore, IgA is thought not to utilize the classical complement system (Ishizaka *et al.*, 1966), though an alternative pathway involving one component of complement, C3, has been demonstrated (Lachmann & Nicol, 1973).

The fact that the vasculitis is confined to the skin in hyperglobulinaemic purpura suggests that local cooling may play a role in its pathogenesis. The production of purpura may be analogous to the skin lesions in patients with mixed cryoglobulinaemia who form large precipitable complexes in the superficial skin vessels on cooling (Meltzer *et al.*, 1966).

Finally, the occurrence of repeated thrombo-embolic episodes has not been reported before in this condition. It is interesting to note that immune complexes have been shown to be capable of activating Hageman factor and thus initiate clot formation (Becker, 1971), though clearly there are many other possible predisposing factors for the development of pulmonary emboli.

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Experiments with human papilloma virus in cell culture

HEATHER A. CUBIE

Department of Dermatology, Royal Infirmary, Edinburgh

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SUMMARY

Monolayer cell cultures infected in the usual way with human papilloma virus produced no significant changes. However, when a nucleic acid extract of the virus was used and the cultures were incubated at a lower temperature (33°C), perinuclear fluorescence was observed 6 weeks after inoculation.

Although the virus of human warts was one of the first to be seen electron microscopically (Strauss *et al.*, 1949) and although many physical properties of the virus have been studied in detail, numerous attempts to obtain an assay system in cell culture (summarized in Rowson & Mahy, 1967) have been generally fruitless. A recent detailed report involving the study of human papilloma virus in known papova virus systems also yielded essentially negative results (Butel, 1972). Several rather similar results have been carried out in this laboratory and are reported here for completeness.

METHODS AND RESULTS

Monolayers of Hep-2 cells incubated at 37°C and inoculated with extracts of human wart virus prepared from parings of untreated simple plantar warts (Cubie, 1972a) showed cell rounding and an increase in pH of the culture medium within 24 h. By 3–5 days some of the remaining cells were enlarged and multinucleate. It required at least 10^9 physical particles in the inoculum for such changes to be easily recognized. Lower dilutions produced a more transitory effect. Similar changes, but to a lesser extent, were observed in rhesus monkey kidney cultures, while secondary human amnion cells showed no changes. These 'cytotoxic' effects were not indicative of a complete virus replication cycle since they could not be passaged. However, while the effects could not be inhibited by specific immune serum raised in rabbits, they appeared to be induced by the virus itself since such large numbers of particles were required and since a similarly prepared extract of plantar calluses produced no such changes.

Fibroblast cultures of human fetal lung, fetal skin and child's skin generally showed no response to inoculation of intact virus under various adsorption conditions of time and temperature. However, on a single occasion, stimulation of growth in the infected tubes of a sparse and degenerating fetal lung culture was observed. Since this occurred in all the infected tubes of this experiment, batch to batch variation was excluded. Using the techniques of acridine orange staining, indirect fluorescent antibody staining with serum from rabbits immunized against human wart virus (Ogilvie, 1970) and autoradiography with stationary cells exposed to tritiated thymidine, no evidence of viral replication or of virus-specified antigens was detected in any experiment with human fibroblasts. Furthermore, no evidence of transformation, as detected by the development of viable colonies in agar suspension (Macpherson & Montagnier, 1964) was ever obtained, even when skin fibroblasts from a mongol child were used (Aaronson & Todaro, 1968).

Negative results in these experiments could have been due to inadequate adsorption of human wart virus to the surface of cells in culture, and indeed, electron microscopical examination of cells (a technique of limited validity because of the large sampling error) and fluorescent antibody studies in the hours following infection showed no definite uptake of virus. Attempts to alter membrane permeability by the addition of the polycation, diethyl–aminoethyl dextran (DEAE–dextran) to the cell cultures failed to aid viral uptake.

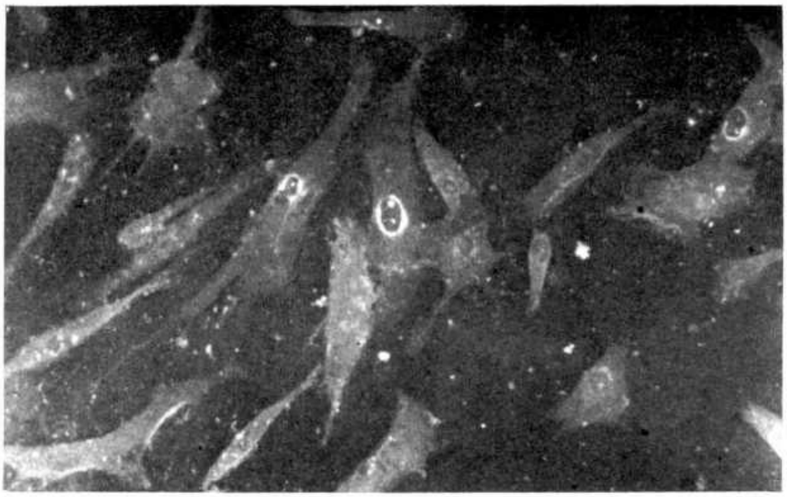

FIGURE 1. Fluorescent antibody stained human foetal skin cells infected 6 weeks previously with nucleic acid from human papilloma virus.

However, encouraging results were obtained when the nucleic acid of a sucrose-purified human papilloma virus extract containing $1{\cdot}2 \times 10^{12}$ particles per ml was used. The virus suspension was pretreated with pronase and the nucleic acid extracted with phenol in the cold (Cubie, 1972a). A 0·1 ml inoculum containing approximately 20 μg DNA and 25 μg DEAE–dextran in Tris/Eagle's minimum essential medium (MEM) (Pagano, 1969) was left in contact with semiconfluent monolayers of Hep-2 and human fetal skin cells grown on coverslips, for 30 min at room temperature. After thorough rinsing with Dulbecco's balanced salt solution, the cultures were incubated in a stationary position at 33°C, in Eagle's MEM containing 5% calf serum and 10% tryptose phosphate broth; the medium being changed at weekly intervals (Cubie, 1972a). Using the fluorescent antibody technique with antibody-containing serum from a student who had had repeated attacks of plantar warts (Cubie, 1972b) and conjugated sheep anti-human globulin, a very bright nuclear ring was first detected in small patches of both types of cells 6 weeks after inoculation (Fig. 1). The appropriate controls with negative serum and uninfected cells were obtained. Fluorescence was still present but had not increased significantly at 7 and 8 weeks after inoculation. No attempts to passage the infected cells were made.

Le Bouvier (1965) failed to stimulate the growth of baby hamster kidney cells with the DNA from human papilloma virus and Butel (1972) also found no effects in human embryonic kidney cells even when these were incubated at 33°C. However, the results reported here suggest that the use of nucleic acid extract in conjunction with both a lower temperature and a longer incubation period may enable some steps in the replication cycle of human wart virus to be established. It is an area of research where little work has as yet been done, but which promises to yield more information than traditional cell culture techniques.

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Pharmacology and Treatment

Low dose azaribine in the treatment of psoriasis

PAUL I.DANTZIG, BERNARD McEVOY, JACQUELINE MAURO
AND SYRUS RAYHANZADEH

Department of Dermatology, Albany Medical Center, Albany, New York, U.S.A.

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SUMMARY

Thirteen patients with severe psoriasis were treated with low dose azaribine (125 mg/kg/day) for 8-week periods. Two patients with generalized pustular psoriasis and four patients with psoriatic arthritis had a good to excellent response. Severe neurotoxicity occurred in four patients, requiring lowering of the dose in three and discontinuance of the drug in one patient. Because of these results, azaribine at 125 mg/kg/day cannot be recommended for plaque-type psoriasis, though it is very effective against generalized pustular psoriasis and psoriatic arthritis.

Of the eleven patients with plaque psoriasis, seven had a good or excellent response initially but subsequently relapsed while on therapy; the other four patients failed to respond to the medication.

During the past decade, cytotoxic drugs have been the most effective form of therapy in severe psoriasis. The most potent of these agents is methotrexate (Van Scott, Auerbach & Weinstein, 1964; Rees *et al.*, 1967; Roenigk, Fowler-Bergfeld & Curtis, 1969; Weinstein & Frost, 1971) but use of this medication has been severely inhibited by its hepatic toxicity (Coe & Bull, 1968; Muller, Farrow & Martalock, 1969; Roenigk *et al.*, 1971; Weinstein *et al.*, 1970). Recent studies on hepatotoxicity secondary to methotrexate show that hepatic necrosis and cirrhosis can occur insidiously without enzyme elevation and may be detectable only by liver biopsy. It is now recommended that methotrexate be used in patients with abnormal pre-methotrexate liver biopsies, excluding simple fatty infiltration, only when the therapeutic need is overwhelming and that these patients be re-evaluated periodically with liver biopsies (Roenigk, Maibach & Wernstein, 1973).

In an attempt to find a less toxic drug for psoriasis, hydroxyurea has also been used (Moschella & Greenwald, 1973). Its toxicity is much less than that of methotrexate, being mainly haematological, but it is also much less effective than methotrexate.

The most recently developed drug for psoriasis is azaribine (Triazure). Azaribine is the orally absorbable triacetyl derivative of 6-azauridine. It is converted to 6-azauridine *in vivo* and 6-azauridine, when converted to its 5'phosphate by uridine kinase, inhibits orotidylic acid decarboxylase, a key enzyme in the *de novo* biosynthesis of pyrimidines (Handschumacher *et al.*, 1962; Creasy *et al.*, 1963; Cardoso, Calabresi & Handschumacher, 1961). This biochemical pathway is greatly increased in neoplastic cells and because of this, azaribine has been noted to have a preferential effect on abnormal proliferating cells while retaining little or no activity against normal cells. Azaribine was first used for the treatment of leukaemias and lymphomas and in 1964, Turner & Calabresi reported the effect of 6-azauridine in psoriasis. Since then, azaribine has been used in two small clinical trials (Calabresi & Turner, 1966) at a dose of 200–300 mg/kg/day and in 1970, Volger & Olansky reported a double blind study of twenty-two patients using 200 mg/kg/day. The majority of patients in each series had a good to excellent response. However, at a dose of 200–300 mg/kg/day, the incidence of neurotoxicity was quite high and most patients required hospitalization for treatment. Central nervous system toxicity included lethargy, dizziness, hyperreflexia, tremor, diplopia, expressive aphasia and coma. In an effort to reduce neurotoxicity and retain efficacy of the drug, a trial of azaribine at 125 mg/kg/day was begun on patients with severe psoriasis.

MATERIALS AND METHODS

Selection of patients

Thirteen patients with severe plaque-type psoriasis, psoriatic arthritis or generalized pustular psoriasis were selected. The patients selected had no systemic illness for which azaribine would be contraindicated, including haematological, renal or neurological disorders. All patients were fully informed about the medication and written permission was obtained prior to therapy. Photographs were obtained prior to and after therapy in most patients. Preliminary laboratory investigations included complete blood count, platelet count, liver function tests (SGOT, LDH, and alkaline phosphatase), renal function tests (blood urea, serum creatinine, and urinalysis), serum iron and total iron binding capacity, uric acid, fasting blood sugar, rheumatoid factor and chest X-ray. Twelve patients were treated as out-patients and one as an in-patient at the Albany, New York, Veterans Administration Hospital.

Plan of study

Patients were treated with azaribine (500 mg tablets), 125 mg/kg/day in four daily divided doses. Treatment was to last 8 weeks unless toxicity required termination of the treatment. Patients were seen at 2-week intervals with a complete blood count and platelet count performed on each visit.

Grading was as follows: 1, excellent—80–100% clearance of skin lesions or absence of joint pain, resolution of joint swelling and full range of motion of the joints; 2, good—60–80% clearance of skin lesions or moderate relief of joint pain and swelling and moderate improvement in range of motion of joints; 3, fair—30–60% clearance of skin lesions or mild relief of joint pain and swelling and mild improvement in range of motion of joints; 4, no improvement—less than 30% improvement in skin lesions or no change in joint symptoms; 5, worse—increase in size and number of skin lesions or increased pain and swelling of joints accompanied by decreased range of motion.

Case material

Thirteen patients, aged 30–67, were selected. Five patients had had previous Goeckerman therapy, eight patients had had previous methotrexate treatment, one patient had received hydroxyurea prior to azaribine, and two patients had received prior parenteral steroids. Eight patients had generalized plaque-type disease, one had generalized pustular psoriasis, one had generalized pustular psoriasis and arthritis and three patients had plaque-type disease and arthritis. One patient had hypertension and was being treated with hydrochlorothiazide and guanethidine. Five patients had a history of more than one drink per day, four stated they had three to seven drinks per week and four patients gave no history of alcoholic intake. Three patients had cirrhosis and one patient had ascites. Four patients had elevation of SGOT and alkaline phosphatase, two patients had elevated uric acid and two patients had mild anaemia.

TABLE 1. Summary of results of azaribine at 125 mg/kg/day in patients with psoriasis

Patient no.	Type of psoriasis and extent of involvement	Response			
		2 weeks	4 weeks	6 weeks	8 weeks
1	Plaque:40%	Good	Worse	Worse	Worse
2	Plaque:75%	Fair	Good*	No change	Worse
	Arthritis:moderate	Good	Good	Good	Good
3	Pustular:100%	Fair	Excellent	Good	Excellent
	Arthritis:moderate	Excellent	Excellent	Excellent	Excellent
4	Plaque:10%	Fair*	No change	Worse	Worse
	Arthritis:severe	Fair*	Good	Good	Good
5	Plaque:40%	Excellent	Good	Worse	Worse
	Arthritis:severe	Excellent	Fair	Good	Good
6	Plaque:75%	Good	Good*	No change	Worse
7	Plaque:80%	Good	Good	Worse	Worse
8	Pustular:100%	Excellent†	—	—	—
9	Plaque:60%	Good	Good	No change	Worse
10	Plaque:60%	Fair	No change	Worse	Worse
11	Plaque:10%	Fair	Fair	No change	No change
12	Plaque:80%	Good	Good	Fair	Fair
13	Plaque:80%	Fair	Fair	Worse‡	—

* Dose reduced to 90 mg/kg/day due to neurotoxicity.
† Drug discontinued due to severe neurotoxicity.
‡ Drug discontinued due to marked flare in psoriasis.

RESULTS

The results are summarized in Table 1. The two patients with generalized pustular psoriasis had a prompt excellent response. However, the patient with generalized pustular psoriasis, in whom azaribine was discontinued due to severe neurotoxicity, remained clear for only 2 weeks after the drug was stopped. The four patients with psoriatic arthritis also had an excellent response with all four patients experiencing relief of pain and joint swelling within 4 weeks. Range of motion was the slowest parameter to respond but all four patients noticed improvement in this within 8 weeks. Psoriatic plaques were the most resistant form of disease. Of eleven patients with plaque-type disease, seven had a good to excellent response initially. However, after 4 weeks of therapy, all seven patients developed resistance to the medication with either worsening of existing plaques or appearance of new lesions. Four patients with plaque-type disease failed to respond to the drug during the entire 8-week treatment period.

TOXICITY

Toxicity from azaribine was troublesome and caused lowering of the dose in four patients and discontinuance of the drug in another (Table 2). Most neurological side effects occurred within the first 2 weeks of treatment. In one patient on azaribine for 10 days, lethargy suddenly developed and progressed to aphasia and a semi-comatose state within a few hours. Despite discontinuance of the drug when lethargy developed, it still required 4 days for the patient to regain alertness and orientation. Two patients complained of severe headaches requiring lowering of the dosage in one and stopping the drug in the other. Another patient developed dizziness and aphasia at 125 mg/kg/day but these

TABLE 2. Summary of toxicity of azaribine at 125 mg/kg/day

Neurological	Haematological	Other
Lethargy (3)	Anaemia (8)	Nausea (2)
Dizziness (3)	Leukopenia (0)	Hypotension (1)
Headaches (2)	Thrombocytopenia (0)	
Aphasia (2)		
Irritability (1)		
Semi-coma (1)		

symptoms subsided when the dose was lowered to 90 mg/kg/day. Three patients complained of lethargy and one of being short-tempered but these were temporary and did not require dosage modification. The patient with hypertension, who was receiving hydrochlorothiazide and guanethidine, developed severe hypotension (80/40 mmHg) which reverted to normal when the azaribine dosage was lowered to 90 mg/kg/day.

Haematological toxicity presented a problem in only one patient. Eight patients experienced at least a 10% drop in haematocrit but none of these patients became symptomatic from the anaemia. One patient, however, had a drop in his haematocrit from 42 ml/100 ml to 24 ml/100 ml during the 8-week treatment period and complained of weakness and lethargy. Three patients had a drop in WBC of at least 3000 cells/mm^3 but no count less than 4000 cells/mm^3 was obtained. There was no significant reduction in platelet count in any patient.

DISCUSSION

Cytotoxic therapy is very important in the treatment and rehabilitation of patients with severe psoriasis and ideally should be of high efficacy and low toxicity so that the patient may be managed outside the hospital. Before starting a patient on one of these drugs, the severity and disability of the psoriasis must be seriously balanced against the potential haematological, hepatic, neurological, teratogenic and immunosuppressive side effects of these agents. Indications for cytotoxic therapy include psoriatic erythroderma, psoriatic arthritis, generalized pustular psoriasis, localized pustular psoriasis, extensive plaque-type psoriasis and psoriasis in areas of the body preventing economic employment.

In a recent study on low dose azaribine in patients with plaque-type psoriasis only, Milstein, Covrell & Stoughton (1973) reported eighteen out of twenty-one patients achieving good to excellent results. Drug toxicity was mild in their study and reduction of the dose by 25% was necessary in only three patients due to depression. Our study, however, was in sharp contradistinction to their results. Patients with severe plaque disease responded very poorly and four of eleven patients had severe enough toxicity to warrant lowering of the dose. These results are also in contrast to favourable reports of the effect of azaribine at a dose of 200–300 mg/kg/day on plaque-type psoriasis (Volger & Olansky, 1970). However, the results in patients with psoriatic arthritis and generalized pustular psoriasis were good to excellent at 125 mg/kg/day, as they have been reported at 200–300 mg/kg/day.

Generalized pustular psoriasis and psoriatic arthritis have a much greater morbidity than plaque-type psoriasis and usually require more aggressive therapy. Low dose azaribine was very effective in pustular psoriasis and psoriatic arthritis in which it appears to be well indicated. However, because of the poor response of patients with plaque disease and the high incidence of severe toxicity, azaribine at a dose of 125 mg/kg/day cannot be recommended for severe plaque-type psoriasis.

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Double blind controlled study of treatment of atopic eczema with a preparation of hydrocortisone in a new drug delivery system versus betamethasone 17–valerate

JOHN ALMEYDA AND B.W.BURT

The Enfield Group of Hospitals, and Chelsea College, University of London

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SUMMARY

A double blind study has shown that topical treatment with a new preparation of 1% hydrocortisone in a stabilized carbamide drug delivery system effectively controls atopic eczema without the hazards of potent fluorinated corticosteroids. Clinical evaluation confirms that it is at least as effective as 0·1% betamethasone 17-valerate. The preparation has the physical properties of both a cream and an ointment and can be used in place of either. It has a high level of patient acceptance and can be used in fissured eczemas without causing any sensations of stinging or smarting.

The introduction of corticosteroids into dermatology resulted in a considerable advance in the management of many dermatoses. Clinical results were improved further when the more potent synthetic fluorinated steroids were introduced. Ease of application and cosmetic acceptability caused patients to accept these preparations readily, and because of their effectiveness in improving symptoms many patients continue to apply them freely, and occasionally excessively.

It is now realized that topically administered fluorinated compounds are just as liable to cause unwanted side effects as are those that are systemically administered

Sneddon (1969) has drawn attention to the effects of fluorinated compounds on rosacea patients, who seem to become addicted as the steroids initially suppress the soreness and inflammatory papular and pustular lesions. However, each time application is stopped the rash exacerbates acutely, tempting the patient to apply more steroid. With prolonged application loss of dermal connective tissue occurs, leading to exposure of the sub-papillary venous plexus with increasing erythema and telangiectasia. The process is slow and the patients do not notice the deterioration in their appearance for some time. The rosacea-like condition, peri-oral dermatitis, may well be due to the use of fluorinated steroids on the face (Weber, 1972).

Potent topical steroids also produce striae (Epstein, Epstein & Epstein, 1963), and extensive bruising and ecchymoses with minor trauma (Burry, 1973) due to wasting of dermal connective tissue. Extreme soft-tissue atrophy in the napkin area in an infant treated for napkin rash with fluorinated steroids for some months has also been reported (Johns & Bower, 1970). Growth of vellus hair, delayed healing of ulcerated areas and systemic absorption with depression of endogenous corticosteroid secretion have all been observed with potent fluorinated steroids (Scoggins & Kligman, 1965; Feiwel, James & Barnett, 1969).

The masking of infection has been well documented (Burry, 1973) and in the case of fungus infections the clinical appearances are frequently so altered and atypical that the diagnosis is easily missed. Moreover, these steroids would appear to perpetuate fungal infections. This state of affairs has become sufficiently common to warrant a title of tinea incognito (Ive & Marks, 1968).

This study was conducted in an effort to produce an effective treatment for various dermatoses free from the side effects of the more potent fluorinated compounds. Hydrocortisone is a non-fluorinated 17-hydroxy corticosterone, that is still widely used in the treatment of many dermatoses. It is not known to produce the side effects of the fluorinated compounds. However, it has been shown to be less effective in the treatment of atopic eczema than 0·1% betamethasone 17-valerate (Williams *et al.*, 1964). The object of this study was to improve the activity of hydrocortisone by devising a different drug delivery system. The system devised relies on the proven ability of carbamide (urea), a natural body constituent, to hydrate the skin and thus, like occlusive dressings, to enhance the penetration of otherwise poorly absorbed medicaments (Swanbeck, 1968). To obtain this drug delivery system, a hypertonic solution of carbamide is loosely bound to an insoluble matrix which consists largely of an inert polysaccharide material. The carbamide is stable in this form and readily diffuses into the skin where it raises the water holding capacity of the epidermis. The hydrocortisone is freely suspended in a non-aqueous medium into which the powder-matrix is evenly dispersed. The system is entirely free from preservative, and trials conducted in an earlier study (to be published) have shown that it is highly effective in the treatment of ichthyosis and does not cause smarting or stinging even when applied to excoriated skin. The method was to employ it to maximise the skin penetration of the hydrocortisone and to assess the result against a potent fluorinated compound (betamethasone 17-valerate) in a double blind controlled study on patients with atopic eczema.

MATERIALS AND METHODS

Thirty-six patients with atopic eczema participated in the trial. The severity of their atopic eczema was graded on clinical assessment as severe, moderate or mild. All patients showed roughly equal involvement on both sides of their body, and also served as their own controls. One week before the trial began, the patients were instructed to discontinue all topical treatments.

Each patient was allocated two tubes of cream, one contained 1% hydrocortisone (UHc powder-cream) the other 0·1% betamethasone 17-valerate. One tube was marked for left side and the other for right side application. The identity of these tubes was unknown to the clinician, and application was randomized so that some treated their left sides with UHc powder-cream and some their right sides. Creams were applied three times daily for 2 weeks, and in a few instances for 4 weeks.

At the end of these periods, patients were re-examined and their clinical condition assessed as excellent, good, no improvement or deterioration. The results were recorded as 'excellent' if the eczema had cleared completely, and 'good' if partially cleared. The patients were also asked to comment on the acceptability of the two treatments.

Five patients also had ichthyosis, and a further four patients also presented dry fissured hand or foot eczema. The original eczema was severe in twenty patients, moderate in six and mild in ten; twenty-six patients had been treated with topical steroids previously. Eighteen patients were male and eighteen female and their ages ranged from 18 months to 74 years; fifteen patients were under twelve years (Table 1).

TABLE 1. Patients and initial state of lesions

No. of patients	Male	Female	Initial state of lesions		
			Severe	Moderate	Mild
36	18	18	20	6	10

CLINICAL ASSESSMENT

After 2 weeks

Eleven patients showed greater improvement with the UHc powder-cream, five with betamethasone 17-valerate. Seventeen patients showed equal response to both preparations. The state of the lesions is described in Table 2.

TABLE 2. State of lesions after two weeks' treatment

	Excellent	Good	No improvement	Deterioration
UHc powder-cream	19	14	3	0
Betamethasone	15	15	6	0

The six patients who showed no response either to UHc powder-cream or to betamethasone 17-valerate were asked to continue application for a further 2 weeks. In addition another group of six who showed good response to both preparations were given the same instructions.

After 4 weeks

The patients were re-examined and of the six who earlier showed no response to either preparation, only one failed to respond to both preparations, while two did not respond to betamethasone 17-valerate. Of the group of six who previously had shown equally good response to both preparations, the improvement was maintained in three, whilst two showed excellent response with both. The overall results are shown in Table 3.

TABLE 3. State of lesions after four weeks' treatment

	Excellent	Good	No improvement	Deterioration
UHc powder-cream	25	10	1	0
Betamethasone	18	16	2	0

Patients' assessment

Fourteen patients commented that they preferred the UHc powder-cream because they thought it was cosmetically superior and seemed to clear the rash more rapidly. Statistical evaluation of the clinical assessment shows that there is a trend in favour of the UHc powder-cream.

DISCUSSION

This study shows that in the treatment of atopic eczema a new preparation of a non-fluorinated steroid, which relies on a stabilized carbamide drug delivery system to improve skin penetration, is at least as effective as a fluorinated compound (betamethasone 17-valerate) in twenty-nine out of thirty-six patients. In twelve patients improvement was more marked with the UHc powder-cream compared with five patients who showed greater improvement with the fluorinated steroid. Of the twelve patients who responded better with UHc powder-cream the eczema cleared completely in nine cases. The initial state of lesions was classed as severe in eight and moderate in four. Of the five patients whose lesions cleared completely with the fluorinated steroid and partially with UHc powder-cream, four were initially severe cases and one moderate.

It is well known that a non-fluorinated is generally less effective than a fluorinated steroid in the treatment of many dermatoses, especially atopic eczema. However, the drug delivery system devised in the present trials increases the activity of a non-fluorinated compound by improving its skin penetration through the well known effect of hydration. In a previous trial (Almeyda & Fry, 1973), using a simple formulation of hydrocortisone and carbamide in a buffered cream, it was shown that the results obtained against betamethasone were comparable. However, a number of patients complained of stinging and smarting sensations following application. This was attributed to the high acidity necessary for stabilizing such a preparation. The drug delivery system devised in the current trials does not provoke stinging or smarting sensations even when applied to fissures or broken skin. This is because the form in which the carbamide is stabilized enables the cream to be kept at skin pH. Since patients with atopic eczema frequently have fissured or excoriated skin, it is advantageous to have a preparation that is cosmetically acceptable and does not irritate broken or inflamed skin.

In appearance and texture the UHc powder-cream is somewhere between a cream and an ointment, and has the properties of both. Therefore, it can be used in conditions which call for either an ointment or a cream. This not only offers some convenience to the prescriber but also renders it more acceptable to the patient. The high level of patients' preference for the UHc powder-cream can only enhance the effectiveness of a treatment where emotional factors may be present.

Side effects

No side effects were observed with either preparation during the course of the trials. However, in view of the small number of patients and the comparatively short period of time during which the subjects were observed, it is not surprising that no side effects were seen with the fluorinated steroid. Nevertheless, past experience has indicated the hazards which can accompany the use of fluorinated compounds.

The UHc powder-cream is a new preparation of a less potent compound which has not been found to have the undesirable side effects of the more potent steroids. The current trials show that it is possible to improve the activity of a less potent compound by utilizing a new drug delivery system. Therefore, in the treatment and control of atopic eczema the UHc powder-cream is a suitable alternative to the fluorinated steroids, being at least as effective but possibly without the hazards of the more potent, fluorinated steroid.

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Case Reports

Concurrent pityriasis rotunda and acquired ichthyosis with IgG myeloma

JUNJI IKADA AND MORIO OKI

Department of Dermatology, Kyoto Prefectural University of Medicine,
Kawara-machi, Hirokoji, Kamikyo-ku, Kyoto, Japan

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SUMMARY

A case of concurrent pityriasis rotunda and acquired ichthyosis associated with IgG-myeloma is described.

CASE REPORT

A Japanese woman, aged 58 years, had had nausea and vomiting for 2 years. She was admitted to the department of medicine on 7 October 1968 and diagnoses of IgG-myeloma, hepatic cirrhosis and urinary tract infection were established. On 14 November prednisolone was started in doses of 200 mg/day and gradually decreased so that by the end of January 1969 she was taking 10 mg/day. On 22 January 1969 she was referred to our department because of an asymptomatic circular eruption of at least 5 days' duration on the abdomen and also scaling of a few weeks' duration on the trunk and limbs. Examination revealed five circular, sharply-defined brown patches with scales and studded follicular papules on the right side of the abdomen. They were partly confluent and darker than the surrounding scaly skin, and their size varied from 2 to 10 cm in diameter. On the trunk and limbs, especially the extensor aspects, diffuse scaling was present. The scales were small and white. Microscopic examination for fungi was negative. A biopsy was taken from a patch on the abdomen. The horny layer was slightly thickened and the follicular orifices were plugged with a horny mass. The granular layer was reduced and the Malpighian layer was flattened. The dermis showed no perivascular infiltrate.

DISCUSSION

Both pityriasis rotunda and acquired ichthyosis may develop in patients with a malignant tumour, tuberculosis, leprosy, or in nutritional deficiencies, and Ito & Tanaka (1960) considered pityriasis rotunda aetiopathologically to be a form of acquired ichthyosis. However, between the two dermatoses a discrepancy in the associated malignant tumours was noted: all of the fourteen patients in whom pityriasis rotunda was a cutaneous marker of internal malignancy had a carcinoma (including malignant melanoma), whereas acquired ichthyosis is uncommonly associated with carcinoma and is seen more with reticuloses. The present case is, to our knowledge, the first to be reported of pityriasis rotunda associated with a reticulosis. Moreover, pityriasis rotunda and acquired ichthyosis developed almost concurrently, and showed a histological similarity. These observations support, therefore, the view of Ito & Tanaka (1960). Furthermore, in addition to multiple myeloma, hepatic cirrhosis probably favoured the development of the two dermatoses.

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A leukaemic acute lymphoblastic leukaemia presenting with cutaneous lesions

T.COCHRANE AND J.A.MILNE

Ayr County Area and Glasgow Royal Infirmary,
and University Department of Dermatology, Western Infirmary, Glasgow

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SUMMARY

A middle aged female patient presented at a skin clinic with a localized subcutaneous tumour of the chest wall. The skin over this showed purpura. Biopsy of this lesion showed it to be a malignant lymphoid neoplasm of lymphoblastoma type. Aberrant lymphoid cells were noted in the cutaneous blood vessels but examination of the peripheral blood at that time showed no evidence of leukaemia. Three months elapsed before a fall in haemoglobin and platelets was recorded and sternal marrow examination confirmed the diagnosis of acute lymphoblastic leukaemia.

CASE REPORT

The patient, a 63-year-old married woman, presented on 3 May 1973, with a 4-month history of a slowly increasing bruise and painless lump on the anterior aspect of the upper left chest. She denied any other symptoms but stated that she had been feeling slightly 'off colour' for the previous few weeks. On examination there was a freely mobile, infiltrated, doughy, subcutaneous tumour 4 cm in diameter in the upper left chest 10 cm below the clavicle. The skin overlying the centre of the lesion contained a small area of haemorrhage and there was a halo of purpura surrounding the tumour. General physical examination was negative; in particular, there was no lymphadenopathy nor hepatosplenomegaly.

INVESTIGATIONS AND PROGRESS

A biopsy of the lesion taken on 8 May 1973 was reported (by J.A.M.) as follows: 'Sections from the skin submitted show diffuse infiltration of the dermis with aberrant cells of the lymphoid series. There are extensive foci of free haemorrhage and many of the blood vessels contain cells similar to those infiltrating the dermis (Fig. 1). We should be interested to know the haematological findings in this patient.'

Haematological examination on 29 May 1973 showed haemoglobin 13·7 g/100 ml, RBC $4{\cdot}5 \times 10^6/\text{mm}^3$, WBC $6{\cdot}6 \times 10^3/\text{mm}^3$, and ESR (Wintrobe) 16 mm/h. A report on the blood film by a haematologist knowing the clinical diagnosis, stated that red blood cells, white blood cells and platelets appeared normal.

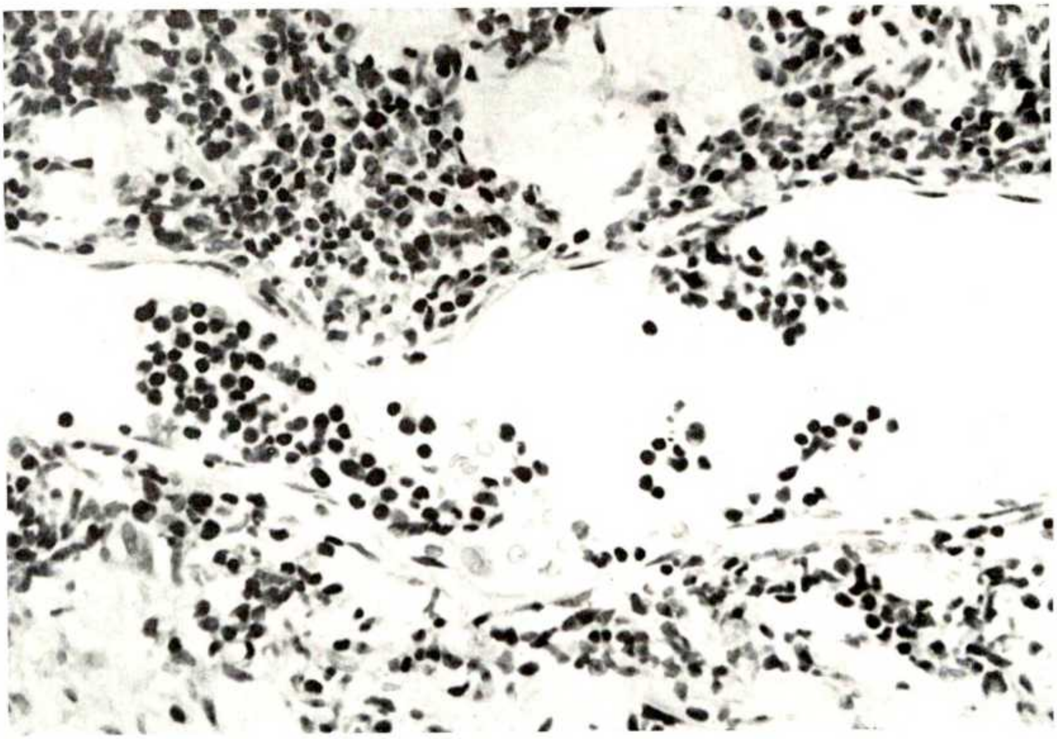

FIGURE 1. Section from original tumour mass to show blood vessel containing aberrant lymphoid cells. The tissue surrounding the vessel is infiltrated by similar lymphoid cells (H & E, × 400).

On this date the patient felt well and the tumour appeared to be slowly resolving. She was seen again by urgent request on 10 July 1973, because 4 days previously she had developed a rash on the upper part of her body. On examination there was a profuse purpuric and haemorrhagic rash on the upper trunk and arms. A few purpuric spots were present on both thighs but the feet, ankles and lower legs were free from lesions. There was a right sub-conjunctival haemorrhage and the cervical and inguinal lymph nodes were moderately enlarged. No enlargement of liver or spleen was detected.

Haematological examination showed haemoglobin 10·4 g/100 ml, RBC $3{\cdot}7 \times 10^6/\text{mm}^3$, WBC $3{\cdot}2 \times 10^3/\text{mm}^3$ and ESR (Wintrobe) 27 mm/h. The peripheral blood film showed slight anisocytosis of the red cells, and platelets appeared reduced. Differential white cell count revealed 39% of blast cells.

Sternal marrow puncture was performed on 10 July 1973, and examination of the material obtained showed widespread infiltration by primitive cells whose nuclei contained one or two nucleoli. These cells were peroxidase negative. The marrow picture was considered to be that of acute lymphoblastic leukaemia.

The patient was admitted to the medical unit of the hospital at this time where she was treated by a regime consisting of vincristine, asparaginase and prednisolone, and when last seen by T.C. (23 July 1973) the lymphadenopathy had decreased considerably and the rash had almost completely faded. On 1 August 1973, she suddenly perforated a duodenal ulcer. This was managed conservatively by drip and suction and she improved until 10 August, when she developed pyrexia and tachycardia. This was diagnosed as being due to a subphrenic abscess and appropriate therapy instituted, but the patient died suddenly and unexpectedly. The autopsy diagnosis was generalized peritonitis, perforated duodenalulcer, lymphatic leukaemia and bronchopneumonia.

DISCUSSION

While skin lesions preceding the onset of leukaemia have been recorded (Wintrobe & Mitchell, 1940) and purpura may be the presenting feature of an aleukaemic leukaemia (Wintrobe, 1967), the present case is unusual in that the cutaneous blood vessels in the presenting dermal tumour contained abnormal lymphoid cells although subsequent peripheral blood examination failed to reveal any evidence of leukaemia; in fact this was not detected until 3 months later.

The presence of the abnormal lymphoid cells within the blood vessels of the cutaneous deposit could of course be explained by intermittent leakage into the vessels from the tissue spaces and thus need not represent a true leukaemia. The main reason for recording this case however lies in the fact that recent advances in the therapy of leukaemia, particularly the acute lymphoblastic type (Spiers, 1972), indicate that the earlier therapy is commenced the better the outlook. In retrospect we wonder, in the light of the biopsy findings, if sternal marrow examination should have been performed which possibly would have allowed treatment to have been started 3 months earlier.

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A painful bleeding syndrome associated with increased fibrinolytic activity

N.R.ROWELL

Department of Dermatology, University of Leeds

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SUMMARY

Two patients with painful bruising and haemorrhagic episodes have been described. Their only haematological abnormality has been an increase in fibrinolytic activity. Each was helped by epsilon aminocaproic acid. This appears to be a new syndrome.

Recurrent painful ecchymoses occur in a variety of conditions. These include autoerythrocyte sensitization (Gardner & Diamond, 1955; Hersle & Mobacken, 1969), sensitivity to DNA (Levin & Pinkus, 1961; Chandler & Nalbandian, 1966), red cell stroma or lipid (Groch *et al.*, 1966), or to haemoglobin (Kremer *et al.*, 1967). It can also occur in factitious purpura (Levin, Chodosh & Sherman, 1969; Stefanini & Baumgart, 1972). Two cases are presented in this report which did not have any of these causes but were associated with increased fibrinolytic activity, an association not previously reported.

CASE REPORTS

Case 1

In 1943, a married woman, aged 33, developed a painful swelling of the left elbow, the skin over which became black. Four weeks later she had further similar trouble which led to a bursa being excised and this was found to be haemorrhagic. Whilst in hospital redness and swelling of the left knee occurred and, later, the right one was similarly affected. A bursa in the region of the right knee was excised at that time and the following year a bursa, also haemorrhagic, was excised from the region of the left knee. In 1949, she had urticaria and, in 1956, following a knock, she developed a haemorrhagic bursitis of the right elbow, and the bursa was excised. Two days later she had an episode of urticaria of the right leg and later of swelling of the face. No precipitating factor was found and she was treated with antihistamines. In 1965, the distal interphalangeal joint of the right index finger became swollen. In June 1966, she developed pain in the region of the ischial tuberosities and when she was referred in November 1966, at the age of 56, she could not sit down because of pain and tenderness in this area. There were no precipitating factors for her symptoms, the time of year, stress or periods appearing to be unrelated. The menses had been regular from the age of 14 to 56. She had noticed intermittent bruising of the skin especially before episodes of bursitis. A female maternal cousin and a niece had had urticaria and a male maternal cousin had had angio-oedema. Her mother had had thyrotoxicosis and a maternal cousin a thyroidectomy.

When first seen in November 1966, she was over-weight and was extremely tender over both ischial tuberosities. There were some freckles on the forearms which did not urticate on rubbing. There was no abnormality of the heart, chest, abdomen or nervous system. The tourniquet test was negative. Her blood pressure was 180/80 mmHg.

Investigations. Haemoglobin 13·4 g%; WBC 5300/mm^3, (neutrophils 57%, lymphocytes 39%, monocytes 1%, eosinophils 3%); platelets 197,000/mm^3; ESR 5 mm/1 h. Serum proteins 6·6 g%, (albumin 4·4 g%, globulin 2·2 g%); serum electrophoresis, normal. WR. and Coombs' test, negative. LE cells, antinuclear factor, rheumatoid factor, cold agglutinins and cryoglobulins, not demonstrated; blood urea 27 mg/100 ml; urine, normal; Serum electrolytes, normal. X-ray of pelvis and right hip showed no abnormality.

The following haematological tests were normal: bleeding time, whole blood clotting time, kaolin cephalin clotting time, prothrombin time, prothrombin consumption index, thromboplastin generation test, thrombin time, Factor VIII assay, Factor XIII assay, anti-activator level, antiplasmin level, plasminogen level and fibrinogen titre. Fibrin degradation products were not demonstrated.

In view of these normal results it was thought that she might be suffering from autosensitivity to erythrocytes but intradermal injections of red cells, DNA, plasma and platelets produced no reaction. In the course of these investigations it was found that this woman consistently had a decreased euglobin lysis time, the significance of which at first was not obvious. This varied from 60–80 min (normal for age more than 120 min).

Treatment. Because of the possibility of an autosensitization type of disorder, she was treated with chloroquine 200 mg twice daily, which had no effect. Later she was given prednisone 5 mg four times daily which appeared to increase her bruising. When the persistently shortened euglobin lysis time was demonstrated she was started on epsilon aminocaproic acid (Epsikapron syrup) 7 g four-hourly, i.e. five times a day. The day after she started this treatment there was a dramatic improvement and she had virtually no discomfort in her buttocks and could sit without a cushion. After about a week she noticed some dizziness, faintness and flatulent dyspepsia and her blood pressure had fallen to 110/60 mmHg. She was kept on this dosage for about 6 weeks when her legs became painful and swollen with pitting oedema and a diagnosis of thrombosis of the inferior vena cava was made. The Epsikapron was stopped and, with rest, postural drainage and supporting bandages, the oedema remitted and did not recur. She was referred to Dr A.A.Sharp at the Radcliffe Infirmary, Oxford, who confirmed the haematological findings including the decreased euglobin lysis time and found a slightly increased plasminogen activator level. For the past 6 years, without any treatment, she has had no further trouble either from bruising or bursitis and her legs no longer swell. Unfortunately she lives in Cumberland and it has been impossible to determine her present fibrinolytic activity as this requires fresh blood taken after fasting.

Summary. A woman with episodes of haemorrhagic bursitis and bruising of the skin, associated with increased fibrinolytic activity, has been described. Permanent clinical remission followed treatment with epsilon aminocaproic acid.

Case 2

A 51-year-old married woman began to get episodes of painful bruising mainly of the arms and forearms in April 1971. The patient was usually aware that bruising was about to occur before there was any objective evidence. The right arm was usually more involved than the left. Episodes sometimes appeared to follow periods of mental stress. No drug could be incriminated. She had had mitral stenosis since the age of 23. There was no relevant family history and, apart from the bruising, general examination showed no abnormality.

Investigations. Haemoglobin 13·7 g%; WBC 6000/mm^3, (neutrophils 38%, lymphocytes 54%, monocytes 4%, eosinophils 4%); platelets 215,000/mm^3; ESR 3 mm/1 h.

The following haematological tests were normal: tourniquet test, bleeding time, whole blood clotting time, kaolin cephalin clotting time, prothrombin time, prothrombin consumption index, two stage prothrombin estimation, thrombin time, antithrombin III, Factor VIII assay, Factor XIII assay, fibrinogen titre, plasminogen, platelet ADP aggregation, platelet factor III availability, and urokinase sensitivity. Intradermal injection of red cells produced no reaction.

Euglobin lysis time 35–70 min (von Kaulla, 1963, as modified by Menon, 1967: normal for age 120 min); serum proteins 7·7 g% (albumin 4·3 g%, globulin 3·4 g%), electrophoresis, normal; serum complement levels, normal; C1 inhibitor, normal. Wasserman reaction, Coombs' test, negative. LE cells, antinuclear factor, rheumatoid factor, cold agglutinins and cryoglobulins not demonstrated.

Blood urea 43 mg%; urine normal; antistreptolysin o titre 1:200; SGOT 12 units; SGPT 8 units; and X-ray chest and electrocardiogram normal.

Biopsy of area of bruising; histologically sections showed an area of fresh extravasation of blood. Some of the surrounding small vessels were cuffed by a few lymphocytes and polymorphs. The overlying epidermis was normal.

Immunohistology of the skin; immunoglobulins were not seen at the dermo-epidermal junction or in the walls of blood vessels but were present in low concentration in the papillary connective tissue.

Treatment. She was treated with epsilon aminocaproic acid (Epsikapron) 7 g by mouth every 4 h for 24 h. This caused nausea and vomiting. A further course was given a week later but had to be stopped after 32 h because of a burning feeling behind the sternum. She had no further bruising for 3 weeks. Over the next 2 years bruising could be controlled by two doses of 7 g of Epsikapron at 12-hourly intervals every 2 weeks, and there were no side effects. Treatment at 3- or 4-weekly intervals was not sufficient to control bruising, which tended to recur in between 3 and 4 weeks.

Forty-two observations of euglobin lysis time were made at weekly or biweekly intervals both before and after the administration of Epsikapron. The levels just before the Epsikapron was given were between 35 and 70 min and, on the day after, between 50 and 144 min (normal > 120 min).

Bruising did not always occur when the blood had a euglobin lysis time between 50 and 90 min but never occurred when the time was above 90 min. The lysis time was always prolonged to some extent within 24 h by Epsikapron.

Summary. A woman with painful bruising syndrome whose only abnormality was a reduced euglobin lysis time. Bruising was controlled by oral epsilon aminocaproic acid given at 2-weekly intervals.

DISCUSSION

Both patients had a bleeding disorder associated with increased fibrinolytic activity as measured by the euglobin lysis time. In neither case was there any evidence of autoerythrocyte sensitization or sensitivity to DNA, red cell stroma or lipid, or to haemoglobin. Although both patients were middle-aged women with excitable personalities there was no evidence that they represented the condition of so-called factitious purpura. There is no identical case in the literature. Reed & Firkin (1957) described a patient with autoerythrocyte sensitization in whom a circulating fibrinolytic factor was present. This was a 40-year-old woman with painful spontaneous bruising and episodes of bleeding after operation or confinement, who had an intracranial haemorrhage. These authors did not estimate the euglobin lysis time but noted break up of the clot and fall out of red cells within 1 h and these changes were almost complete in 12 h. Further experiments suggested that the lytic factor was associated with the red cells and not with the plasma or serum.

Fibrinolysis is a physiological process ensuring that fibrin, which is continually being deposited on vessel walls, is removed. It is essential for the maintenance of the patency of blood vessels. The proteolytic enzyme responsible for fibrinolysis is plasmin which is capable of digesting fibrinogen, Factors V and VII as well as fibrin. Normally plasmin is not present in tissues or body fluids but its precursor, the globulin plasminogen, is widely distributed. The conversion of plasminogen to plasmin is initiated by activators which are present in blood, secretions and tissues, including capillary endothelial cells. The process may be stimulated by intense exercise, mental shock, major surgery, ischaemia, hypoglycaemia or electric shock. Fibrinolysis is controlled by inhibitors which are of two types. Firstly, there are anti-activators such as anti-urokinase, anti-streptokinase and anti-tissue kinases; secondly, there are anti-plasmins. Anti-plasmin is normally in excess so fibrinolysis depends more on the release of plasminogen activator than on the level of circulating free plasmin. Fibrinolysis is also inhibited by epsilon aminocaproic acid which mainly inhibits plasminogen activator.
Excessive fibrinolysis occurs in:

(1) Malignancy such as carcinoma of the prostate (Tagnon *et al.*, 1953), carcinoma of the pancreas (Ratnoff, 1952), giant cell carcinoma of the lung (Davidson *et al.*, 1969) or Hodgkin's disease.
(2) Polycythaemia, leukaemia (Mikata *et al.*, 1959) and cirrhosis of the liver (Grossi, Moreno & Rousselot, 1961; Fletcher *et al.*, 1964).
(3) Shock (Weiner, Reid & Roby, 1950; Reid, Weiner & Roby, 1953; Hodgkinson & Luzadre, 1954).
(4) Mental and physical stress (Sawyer *et al.*, 1960).

All these conditions are probably associated with increased production of activator.

The present cases do not seem to be associated with any of the foregoing disorders. It is postulated that both patients had a fluctuating level of plasminogen activator and localized bleeding occurred into a bursa, a joint or the skin on mild local trauma. The source of the activator is unknown. It is interesting that episodes of bruising in the second patient are always worse when she is mentally agitated and particularly occur in the right arm after exercise.

Epsilon aminocaproic acid specifically inhibits the activators which cause the conversion of the inactive plasminogen to plasmin, by competing with activators for the enzyme binding sites on the plasminogen molecule (Alkjaersig, Fletcher & Sherry, 1959). It is rapidly absorbed from the gut with a peak plasma level 2 h after an oral dose and within 12 h 60–90% appears in the urine (McNicol *et al.*, 1962). It is thus surprising that the bruising in Case 2 can be so well controlled by giving the drug at intervals of 2 weeks. Side effects include nausea and vomiting, diarrhoea, nasal congestion, rashes and transient hypotension, but the most serious is thrombosis. Thrombosis of the inferior vena cava occurred in the first patient after 6 weeks of continuous therapy and this led to the use of interval treatment in the second patient.

The importance of these cases is to draw attention to the necessity of determining the euglobin lysis time in all cases of painful bruising, whether or not accompanied by other bleeding tendencies. Cunliffe (1968) and Dodman *et al.*, (1973) have drawn attention to the decreased fibrinolytic activity in certain cases of vasculitis, and have successfully treated such cases with phenformin and anabolic steroids such as ethyloestrenol or stanozolol. The cases described in this paper represent the opposite situation.

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Comment

THE IMMUNOLOGICAL IDENTIFICATION OF THE SÉZARY CELL

In 1938, Sézary & Bouvrain were first to report a disease characterized by a generalized erythroderma and very large atypical mononuclear cells in the blood.

A year later, Baccaredda described the syndrome under the name of benign hyperplastic cutaneous reticulohistiocytosis with melanodermia. This condition is nowadays referred to as the Sézary syndrome. The disease is most often heralded by the outbreak of dermatitis on the limbs and face; eleven out of nineteen cases reported by Winkelmann & Linmann (1973) began in this fashion. Occasionally contact dermatitis may occur. This early phase is soon followed by a generalized erythroderma with pigmentation, oedema, itching and by hyperkeratosis of the palms and soles (present in 35% of Winkelmann's series). The superficial lymph nodes become enlarged.

Histologically there is a sub-epidermal monomorphous infiltrate made up of atypical lymphocytes and large cells (diameter 15–25 μm) with indented nuclei. In the relatively pure forms of this syndrome, there is no lymphocyte exocytosis within the epidermis and no abscesses as in mycosis fungoides. Tateishi & Wada (1965) have drawn attention to the importance of collagen degeneration in the infiltrated areas. Acanthosis, parakeratosis and hyperkeratosis which may occur in the epidermis are not specific to this syndrome.

The peripheral blood leukocyte count is usually between 15–20,000/mm^3. It is sometimes higher with a lymphocytosis. Eosinophilia is inconstant. Histochemical studies have shown the Sézary cells to contain PAS positive cytoplasmic inclusions and give positive reactions for betaglycuronidase, but alkaline phosphatase and peroxidase stains are negative. The cells do not phagocytose bacteria or latex particles. These properties suggest that the Sézary cell has a closer affinity to lymphocytes than to macrophages or monocytes.

Lymph nodes may be invaded by Sézary cells in a similar way to the invasion of the skin. In the classical case the bone marrow is not involved, which is one of the features that distinguishes the Sézary syndrome from the leukaemias.

Electron microscopy has demonstrated that Sézary cells have a cerebriform nucleus containing homogeneous chromatin and a nucleolus. These appearances are non-specific as normal lymphocytes cultivated with mitogens develop similar characteristics (personal observation).

Cytogenetic studies have shown that the Sézary cell can be diploid, triploid or tetraploid (Crossen *et al.*, 1971). In addition there are different chromosome markers (A, B, M, or G) which suggests that proliferation is not limited to a single cell clone. On this basis, Lutzner (in press) classified the cells into two types, a small diploid cell type with an irregular shaped nucleus and a large tetraploid cell type with a cerebriform nucleus.

It is well known that lymphomas, lymphosarcomas or Hodgkin's disease may arise in patients with the Sézary syndrome (Fleischmajer & Eisenberg, 1964; Lapière & Renkin, 1960), and Winkelmann (1973) considers that the syndrome is a transitional lymphoproliferative disorder that should be classified with the lymphomas and mycosis fungoides.

The disease occurs among the elderly and consequently the survival is often short (2–5 years). However, in females the onset may be earlier and they can survive for many years. Only three of the nineteen patients seen by Winkelmann at the Mayo Clinic died of lymphomas, the remainder died of cardio-vascular disease and infections.

It is only in recent years that immunological studies have contributed to the understanding of the Sézary syndrome. The immunoglobulin levels in the peripheral blood are normal, as are skin delayed hypersensitivity reactions to various antigens (PPD, Candidin, streptokinase-streptodornase, and mumps antigen). Taswell & Winkelmann (1961), on the basis of histopathological studies, drew attention to the probability that the Sézary cell is of lymphoid origin, but it was several years later that its identity was confirmed immunologically.

The normal lymphoid cell population arises from stem cells in the bone marrow, some of which can differentiate in the thymus to give rise to T lymphocytes that participate in delayed hypersensitivity reactions, homograft rejections and graft-versus-host reactions.

Stem cells also differentiate in the bursa of Fabricius, or its equivalent in man, the gut-associated lymphoid tissues (tonsils, Peyer's patches, and the appendix), to give rise to B lymphocytes which initiate the synthesis and secretion of immunoglobulins.

The identification of T lymphocytes is based on their biological properties—blastic transformation induced by specific or non-specific mitogens such as phytohaemagglutinin (PHA) or concanavalin A (Con A), the presence of surface membrane receptors for sheep red blood cells (Froland, 1972; Wybran, Fudenberg & Sleisenger, 1971) and the specific cytotoxic effect of anti-T lymphocyte antisera upon the cells. Negative criteria are important when making a complete study of T lymphocytes. They do not have receptors for immunoglobulins (Unanue, Grey & Rabellino, 1971), antigen-antibody complexes (Nussenzweig, Bianko & Dukor, 1971), or aggregated immunoglobulins (Dickler & Kunkel, 1972).

Crossen *et al.* (1971), Durie & Taswell (unpublished data) have shown that Sézary cells undergo blastic transformation when stimulated by PHA but have a low reactivity in a one way mixed leukocyte culture. Claudy, Ritts & Winkelmann (1973) (unpublished data) observed that the stimulation of Sézary cells was limited to T mitogens (PHA and Con A), without obtaining any reaction to B mitogens such as pokeweed mitogen (PWM).

Sézary cells in skin fragments when cultured with PHA or PWM were only induced to synthesize DNA by PHA. There was a significant increase of the DNA content of the Sézary cell induced by PHA stimulation as indicated by the Feulgen staining. Extracts of skin affected by the Sézary syndrome caused lymphocyte transformation of isologous lymphocytes without any effect on normal lymphocytes (Claudy *et al.*, 1973).

The absence of receptors for immunoglobulins or C3 on the cell membrane is one of the main criteria used to identify a thymus dependent lymphoid cell. Brouet, Flandrin & Seligmann (1973), Broome *et al.* (1973) and Claudy *et al.* (unpublished data) have shown that these receptors do not occur on Sézary cells. Spontaneous rosette formation with sheep red blood cells is another positive feature characteristic of T lymphocytes (Froland, 1972). 65 to 90% of Sézary cells are capable of forming these rosettes (Brouet *et al.*, 1973). Anti-T lymphocyte antisera have been observed to be cytotoxic for most Sézary cells (Brouet *et al.*, 1973). The large and small type of Sézary cell reacted in the same manner. Brouet *et al.* (1973) observed that the numbers of B lymphocytes bearing surface immunoglobulin was very low, 1–4%, but that the distribution of heavy and light chains was normal.

DISCUSSION

In view of these findings it is evident that Sézary cells are related to T lymphocytes. It is possible that those cells which are triploid or tetraploid may arise from immune stimulation, similar to that induced by PHA.

The erythroderma might be due to a cutaneous response to lymphokines (SRF and MIF) secreted by the lymphocytes in the infiltrated areas. It is still an open question whether the Sézary cell is a tumour cell arising from a malignant cell clone or whether it is the product of chronic immunological stimulation.

It has been reported that in mice a chronic graft-versus-host reaction can give rise to a lymphoma (Armstrong *et al.*, 1970). A similar sequence of events may occur in the Sézary syndrome. The inefficiency of immunosuppressive therapy strongly suggests that this may be the basis of the disease.

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Clinique Dermatologique (Prof. J. Thivolet),
Hôpital E. Herriot,
69374 Lyon Cédex 2,
France

A.L.CLAUDY

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Correspondence

'OPPORTUNISTIC' AUTO-IMMUNE DISEASE AND 'OPPORTUNISTIC' MALIGNANCY FROM IMPAIRED CELL MEDIATED IMMUNITY?

SIR, The valuable review by Krain (1974) discusses possible explanations for the association of pemphigus, malignancy and thymic disease. It seems certain that these phenomena are more than coincidentally related and four possible explanations are discussed. May there not be yet another speculative explanation? May not both pemphigus and malignancy be epiphenomena—due to a single underlying cause, namely, T-cell depletion or insufficiency?

Thymic disease—aplasia, hypoplasia, dysplasia and advancing age,—may all interfere with the development or maintenance of cell mediated immunity. With the loss of this surveillance mechanism may not both auto-immune disease, including the pemphigus group and myasthenia gravis, for instance, and malignancy emerge sooner or later? The concept of T-cell control on the synthesis of antibodies by B-cells permitting auto-immune disease was postulated by Allison, Denman & Barnes, (1971) and the same concept was postulated in relation to lymphomata by Gershwin & Steinberg (1973). Of course, we see just the same late developments with certain genetic diseases characterized by defective cell mediated immunity. These include ataxia–telangiectasia, Wiskott-Aldrich syndrome and Chediak-Higashi disease.

Perhaps the analogy to the well-known opportunistic infections is a good one and, indeed, perhaps we should diligently search for and record examples of the combination of opportunistic infections (OI), 'opportunistic' auto-immune disease (OAID) and 'opportunistic' malignancy (OM). Just as in the last decade the clustering and overlapping of syndromes within the auto-immune group of diseases has been recorded and clarified, perhaps now is the time to record the clustering and overlapping between OAID and OM; OI and OM; OI and OAID.

Opportunistic infections include certain recurrent and torpid bacterial infections, widespread candidiasis and dermatophytosis, profuse eruptions of viral warts (including perhaps epidermodysplasia verruciformis) and widespread molluscum contagiosum. Within the herpes group widespread and necrotizing lesions are characteristic, with impaired cellular immunity.

OAID include the organ specific group, particularly the vesiculo-bullous variety, including pemphigus vulgaris, pemphigoid, and the overlapping syndrome of SLE and pemphigus vulgaris (Senear-Usher syndrome). The auto-immune endocrine gland disorders are now well recognized and probably alopecia areata, vitiligo and Sutton's naevus should now qualify for inclusion. The wisespread non-organ specific diseases include S.L.E., rheumatoid arthritis, dermatomyositis, etc.

In the OM group perhaps we should look not only for lymphomata and visceral carcinomata, but cutaneous malignancy—the activation of existing actinic keratoses and the appearance of slowly resolving keratoacanthomas.

We should not expect all cases of pemphigus or, indeed, of malignancy to result from this escape mechanism, any more than all fungal infections depend upon immunological depletion. However, the clinician should be alert to the possibility of the coexistence of OI, OAID and OM, and realize that the primary cause of T-cell depression may not be due to thymic disorder, but might occur in Hodgkin's disease or even sarcoidosis. The problem for the experimentalists is to determine in those cases of auto-immune disease and malignancy whether the T-cell depression is the cause or the result of the disorder; it would be of interest to consider monitoring T-cell function and to consider using transfer factor for immunorestitution.

St Bartholomew's Hospital, Rochester, Kent ROBERT J. CAIRNS

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Book Reviews

Coli: Great Healer of Men. The Biography of Dr Leonard Colebrook FRS. W.C.NOBLE, (1974) London: William Heinemann Medical Books Ltd. Pp. 149, illustrated. Price £1.25.

During a long (1883–1967) and exceptionally productive life Leonard Colebrook made numerous contributions to bacteriology, to immunology in the old and newer senses of this term and to the treatment of infections. He was trained under Sir Almroth Wright and worked mainly at St Mary's Hospital and in various Medical Research Council units at Hampstead, Queen Charlotte's Hospital, the Glasgow Burns Unit and the Birmingham Accident Hospital.

He richly deserved to be the subject of a good biography. His changing interests were directed in turn to many of the outstanding practical problems of each decade, and he made important contributions towards their solution. His was a shy and endearing personality—in his early days he began and ended his journeys for consultations in the country on a bicycle, with his little dog Fitzgoblin in a basket on the handlebars—and his modesty perhaps explains why his great achievements were never acknowledged by the British Honours system.

Dr Noble has had access to Colebrook's diaries and papers and has been helped by the personal recollections of many of his former colleagues and friends. He has used his material with great skill and has written an attractive and very readable biography, easily understood by the layman because technical terms are explained by apt analogies, yet presenting a serious study of the scientific research of a great man, whose personality remains vividly alive throughout the narrative.

Colebrook's earlier work was concerned with a wide variety of subjects ranging from vaccine therapy to skin grafting, and the use of pneumothorax in tuberculosis. He soon became interested in wound infection, his work on which led him to problems of cross infection and the prevention and treatment of puerperal fever. His remarkable work on the latter involved him in some of the earliest trials of the sulphonamides. Later he was chiefly known for his work on the infection of burns and for his further studies of cross infection. However, at no stage of his career did he confine his publications rigidly to any narrow field. In the final years of his active career he campaigned vigorously and successfully for legislation to reduce the risk of burning accidents in the home.

Dr Noble is of course well known as bacteriologist to St John's Hospital for Diseases of the Skin, London, but this alone would not justify the publication here of a review of his book. There is growing interest in the history of dermatology, but too many of its exponents appear to confine their attention to the publications of dermatologists. The study of any speciality with such an outlook is sterile antiquarianism. The history of dermatology is meaningful only if it is related to the advances in the basic sciences on which it depends, and if these in turn are related to the social, economic, and political forces which generate them. Only a few of Colebrook's many papers were concerned with the skin, but this story of his life is recommended to all medical historians including historians of dermatology, and also to those who just enjoy a good biography.

ARTHUR ROOK

Ethnic Pigmentation. H.P.WASSERMANN (1974). Amsterdam: Excerpta Medica. Pp. ix+284, 33 illustrations. Price £12.75.

Dr Wassermann is a member of the Pigment Metabolism Research Unit and of the Department of Internal Medicine at the University of Stellenbosch, South Africa. He has become widely known for his concept that racial differences in pigmentation are not in themselves adaptations to the environments in which they evolved; rather are they the incidental consequence of adaptation of the reticulo-endothelial system to a disease-ridden environment, through decreased adrenocortical activity.

After an introductory chapter, this book begins with an interesting summary of some of the very extensive literature on the nature and significance of racial pigmentation, ranging from the myths of prehistory to contemporary hypotheses. The inadequacy of the latter is emphasized. Increased pigmentation as protection of the skin against damage by ultraviolet irradiation is unlikely to have conferred any selective advantage as it so rarely leads to death before the reproductive period.

Anthropological surveys of skin colour, normal and abnormal, in various ethnic groups, are reviewed and discussed and a well-documented account is given of the physiological and biochemical aspects of pigmentation, and of its control mechanisms. Racial differences in disease incidence are then considered in relation to differences in the metabolism of melanin. The role of melanin in the central nervous system is discussed both as an incompletely solved problem in neurochemistry, and in relation to racial differences in the incidence of the so-called pigmentation–dyskinesia–psychosis triad. A final chapter recapitulates the essential features of the author's concept.

This book is stimulating reading and it leads the dermatologist through much territory which is largely unfamiliar to him. The bibliographies, which support each chapter, are lengthy. One is left with the feeling that Dr Wassermann may be getting near the true solution to a fascinating and fundamental problem in human biology, but that the case is as yet unproven. One great merit of this work is that it puts forward many hypotheses which can be tested by future research, and that much of the essential research—for example, to establish the true incidence in different races of various disorders in which melanin is vitally involved—can be undertaken by those whose limited facilities restrict them to projects which are largely or purely at an observational level.

ARTHUR ROOK

British Journal of Dermatology (1974) **91**, 605.

News and Notices

British Association of Dermatologists. Annual Meeting, Cambridge, July 1975

The Annual Meeting of the British Association of Dermatologists will take place in Cambridge on 3–5 July 1975, under the presidency of Dr Arthur Rook. As an innovation, there will be additional papers of a laboratory orientated nature on the Friday afternoon for those who do not wish to join in the social and sporting activities. The rest of the programme will be as usual.

Papers are invited and should be sent in the form of a summary of approximately 200 words (6 copies) to the Hon. Secretary, Dr J.S.Pegum, The London Hospital, London E1 1BB, before 1 March 1975. If there is more than one author, please underline the name of the speaker. *The British Journal of Dermatology* hopes to publish, in a supplement, the summary of those papers which are accepted. These, therefore, should be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by *The British Journal of Dermatology*.

Advanced Seminar in Dermatology

This Annual Seminar is open to Consultants and Senior Registrars in Dermatology. It will be held from 14–18 April 1975 at the Institute of Dermatology and St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ. Applications should be sent to the Dean before 31 December 1974.

Dermatological Society of South Africa

The Third South African International Dermatological Congress will be held in Durban, from Wednesday, 18 to Saturday, 21 June 1975, at the Blue Waters Hotel.

The main themes of the Congress will include (a) Progress in Dermatological Therapy; (b) Aspects of Tropical Dermatology; (c) Immunology; (d) Cutaneous Malignancy. Other Sessions will be devoted to free (miscellaneous) communications of short duration.

International participation is invited and the official language will be English.

Full details available from Dr B.Bentley-Phillips, 708 Eagle Building, West Street, Durban 4001, South Africa.

FELLOWSHIPS AND AWARDS

Royal College of Physicians

Dr E.N.M.Johnston was inadvertently omitted from the list, published in the July issue, of Dermatologists recently elected to the Fellowship of the Royal College of Physicians, London.

London University Diploma in Dermatology Examination

The following were successful in the Diploma of Dermatology Examination held in July 1974:

Dr A.A.M.Al-Yasiri
Dr L.H.Bestourous
Dr W.J.Burton
Dr F.H.Chowdhury
Dr S.M.Chu
Dr A.A.T.Etemadi
Dr S.Ghussne
Dr R.S.N.Khoo
Dr F.H.Mansouri
Dr Y.M.Olumide
Dr D.M.Owili
Dr S.P.Patel
Dr F.S.Qaqish
Dr S.Qureshi
Dr A.M.Raizada
Dr M.Skeete
Dr C.C.Tanprasert
Dr C.Viravan

A Mark of Distinction was awarded to Drs Burton, Khoo, Raizada and Skeete.

Corrigenda

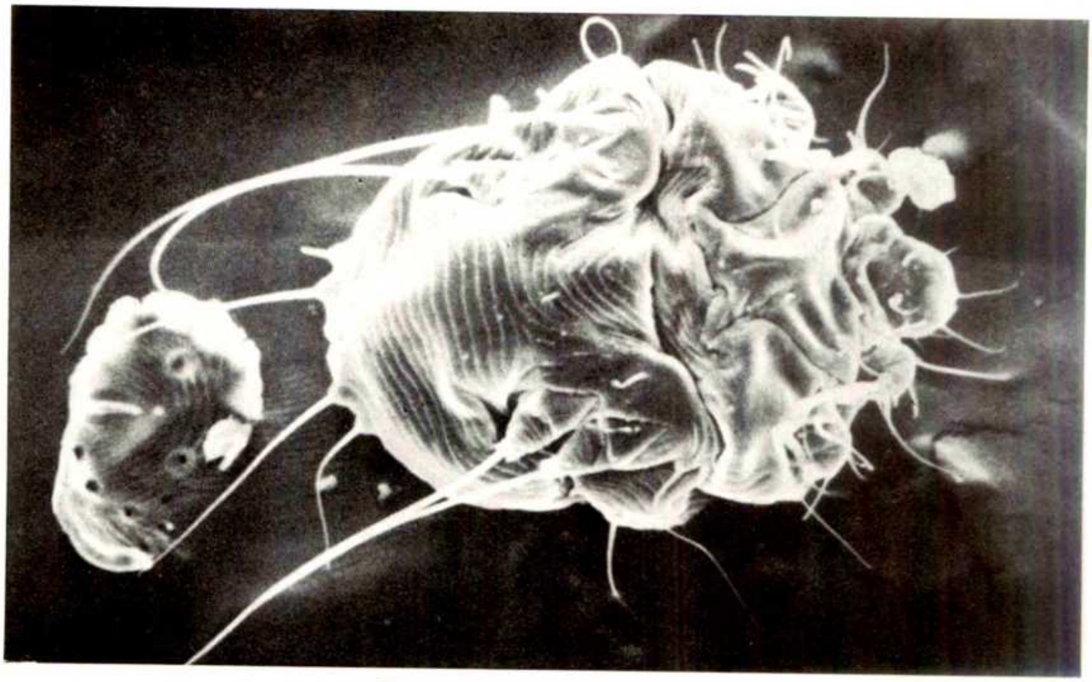
In the paper by R.H.Cormane, S.Husz and F.Hamerlinck, 'Immunoglobulin- and complement-bearing lymphocytes in allergic contact dermatitis and atopic dermatitis (eczema)', which appeared in the June issue of the Journal (Vol. **90**, p. 597) the mean values in Tables 4 and 5 on p. 601 should have read as follows: (apologies—ed.)

TABLE 4

	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%	C3%
Mean value	4·1	1·2	5·0	3·5	2	15·8	6·2

TABLE 5

	IgG%	IgA%	IgM%	IgD%	IgE%	Total Ig%	C3%
Mean value	6·0	1·9	5·5	35·9	4·4	53·2	32·5



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Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P. (Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P. (Lond.)
Robert Harold Champion M.A. M.B. B.CHIR. (Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D. (Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S. (Lond.) F.R.C.P.(Lond.)
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William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
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Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
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Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
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Clinical Tropical Dermatology
Edited by Orlando Canizares M.D. F.A.C.P. Autumn 1974. 700 pages, 272 illustrations (40 colour). About £10.00

Lecture Notes on Dermatology
Bethel Solomons M.A. M.D. F.R.C.P.I. *Third Edition*, 1973. 268 pages, 107 illustrations (37 colour). Paper, £2.25

Manual of Dermatologic Syndromes
Thomas Butterworth A.B. M.D. MED.SC.M. *Second Edition*, 1972. 104 pages. Lippincott, £1.50

Cutaneous Lesions of the Lower Extremities
M.H. Samitz M.D. M.SC.(Med.) and Alan S. Dana Jr M.D. 1972. 210 pages, 132 colour illustrations. Lippincott, £14.00

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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Throughout 1974 the costs of production and distribution have continued to escalate, and an increase in the subscription price is unfortunately essential in order to keep pace with additional costs already incurred and those anticipated during 1975. The rate for Volumes 92 and 93 will be £18.00 per annum, post free (U.S.A. and Canada $63.00, including the cost of despatch by air).

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1. Brit. J. Derm (in press)
2. Archs. Derm. **86**,608,1962
3. Brit. J. Derm. **90**, 197, 1974

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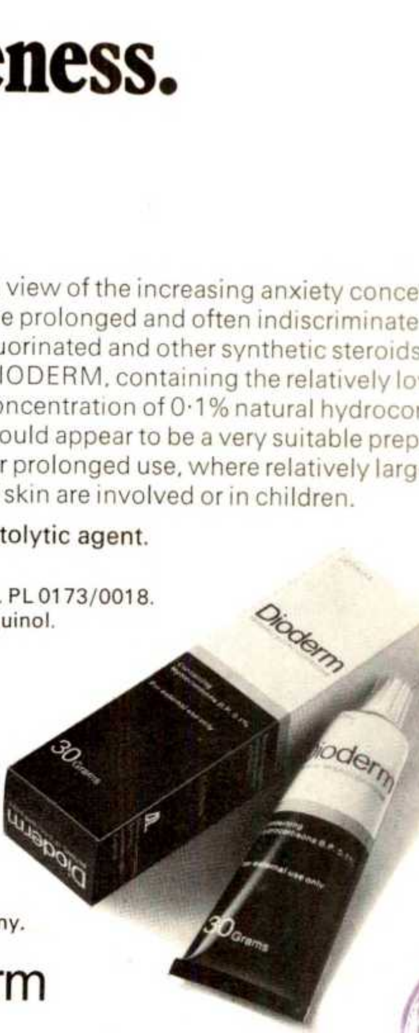

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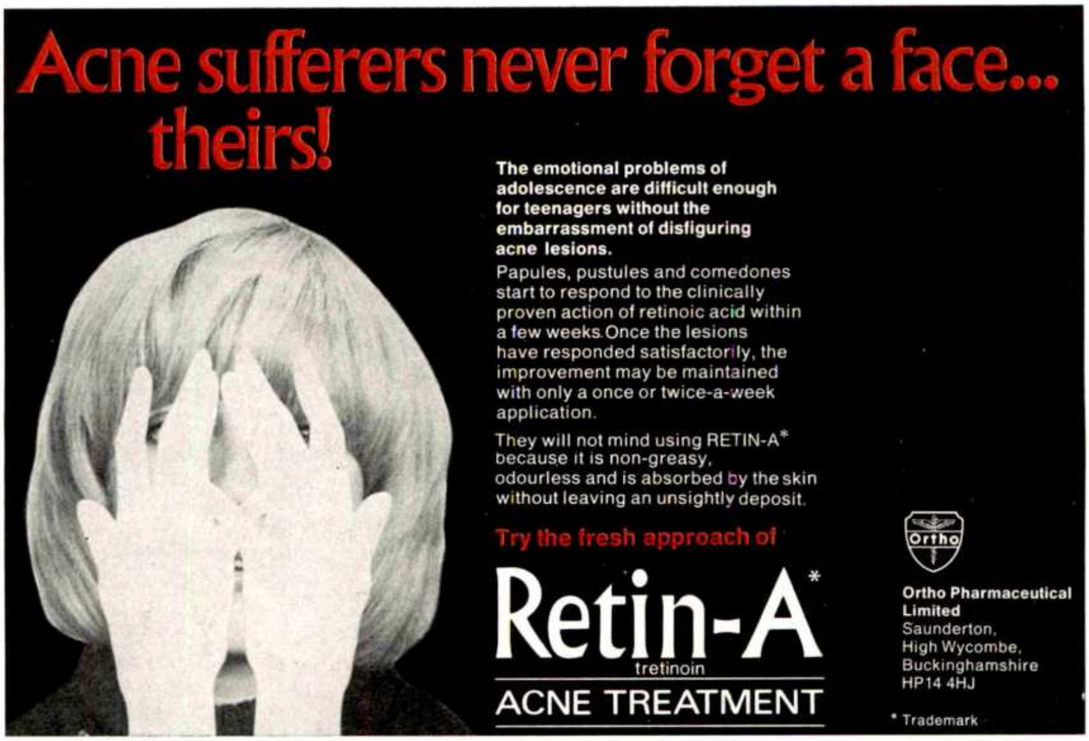
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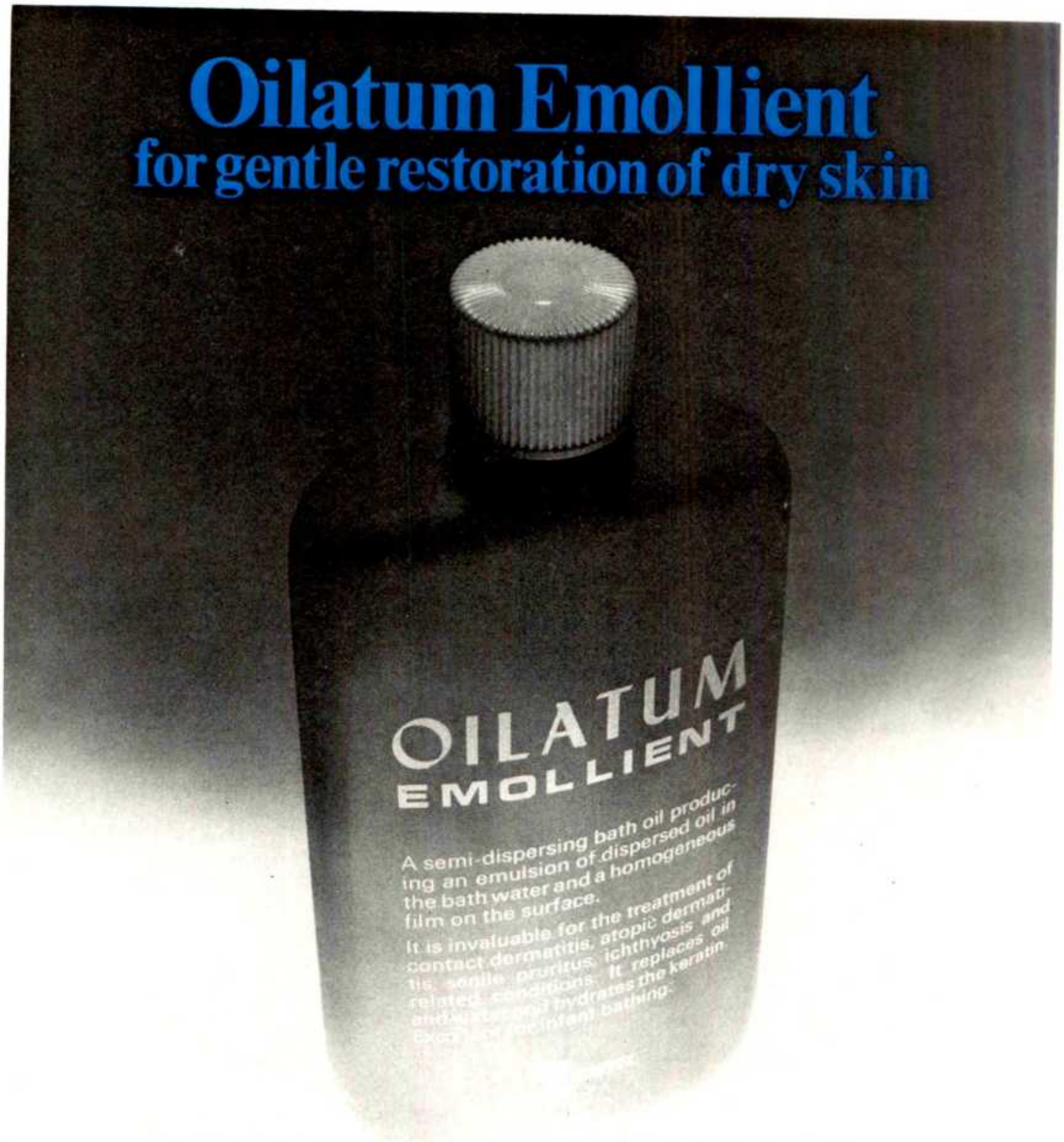
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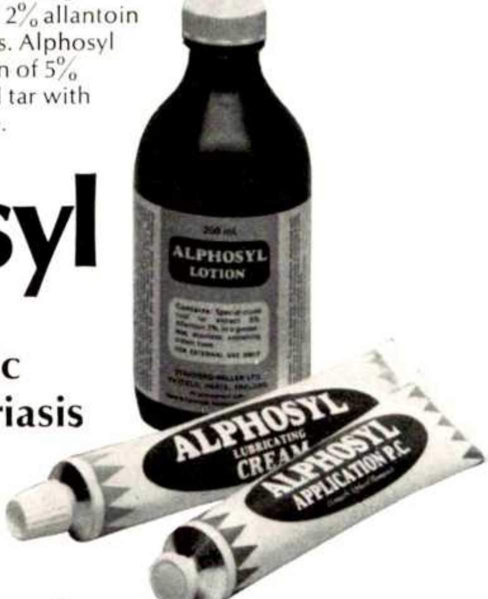

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2. *Brit. J. Derm.* (1968) **80:** 791.
3. *Practitioner* (1970) **204:** 625.
4. *Brit. J. Derm.* (1965) **77:** 163
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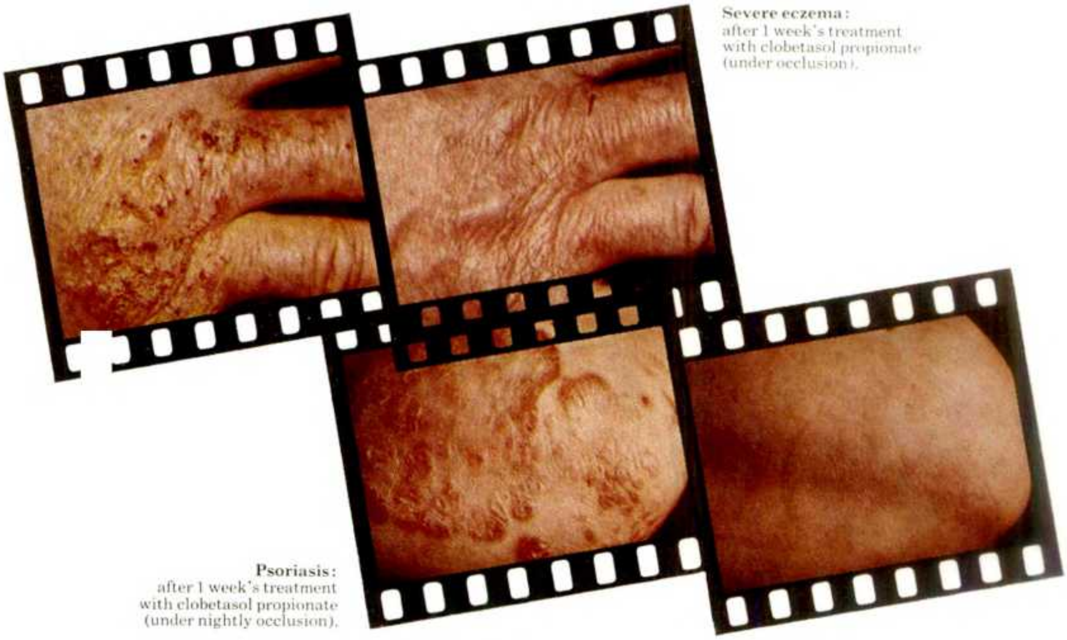

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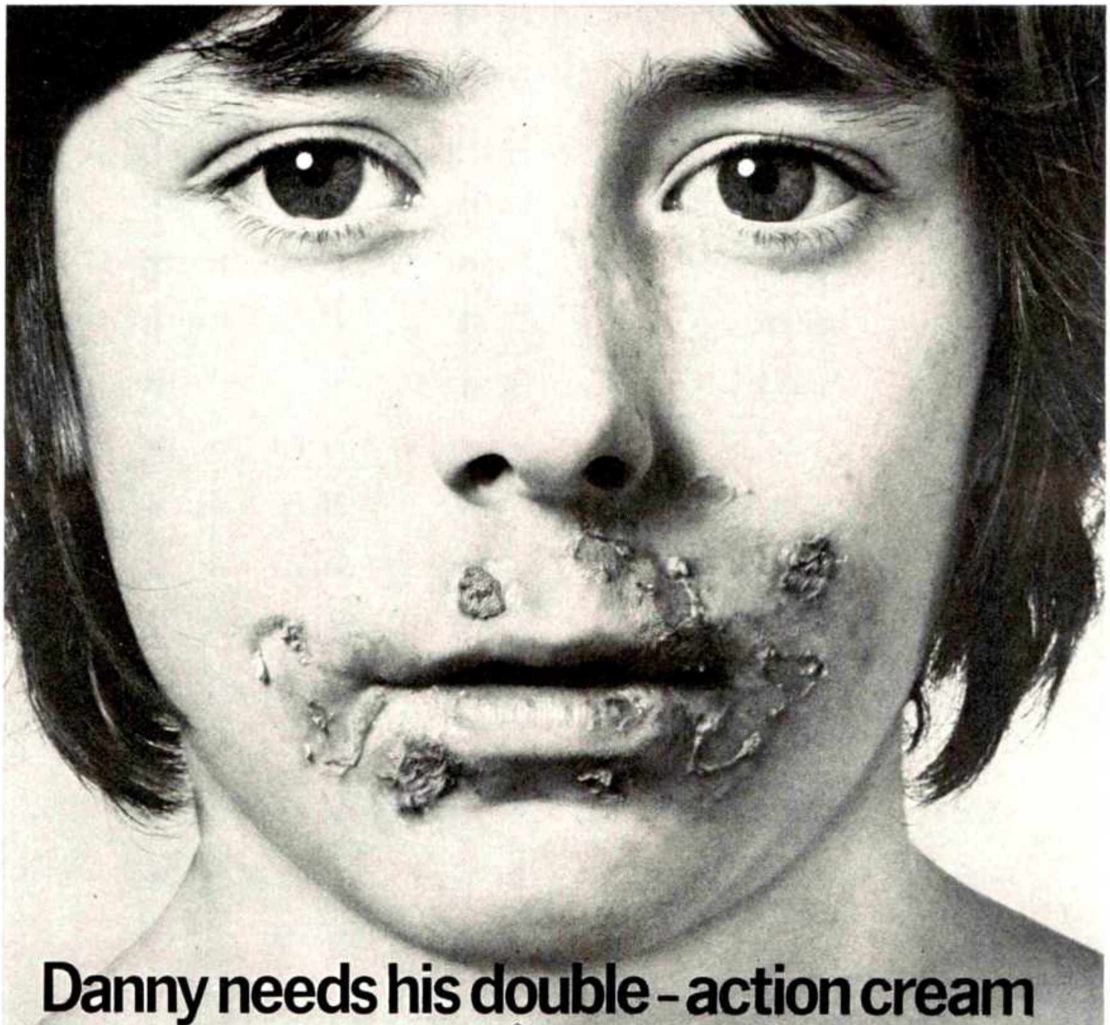

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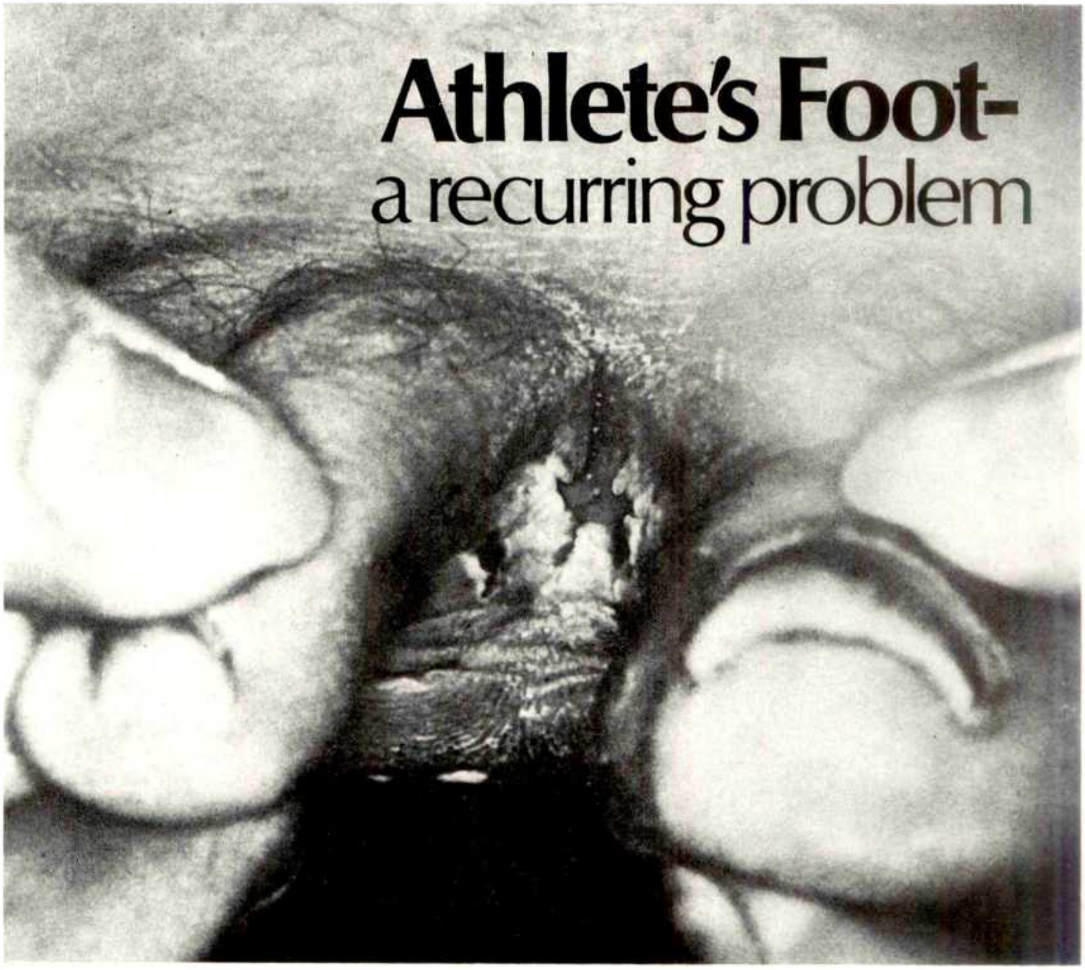

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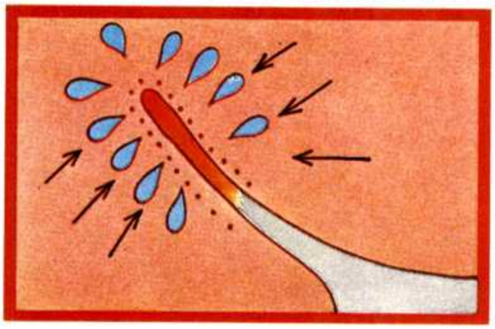
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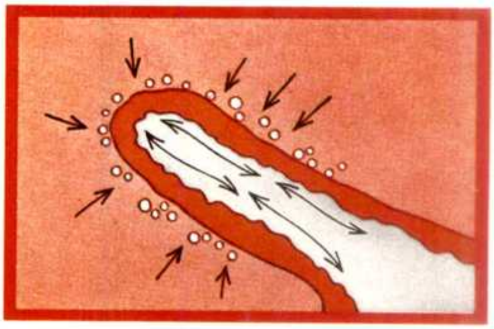
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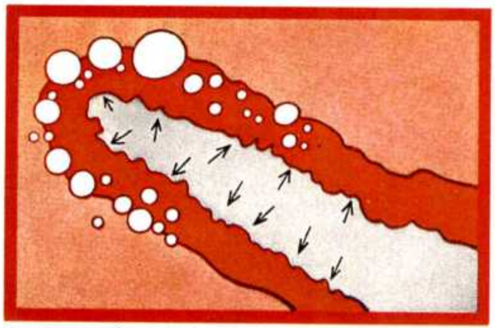
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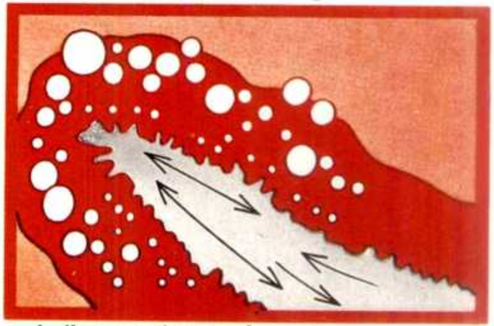
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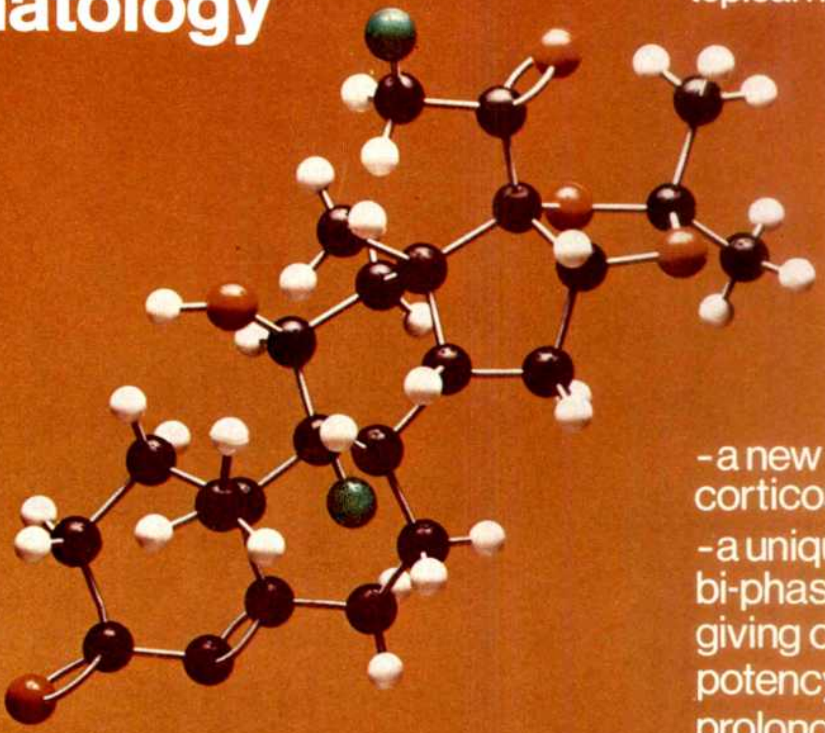

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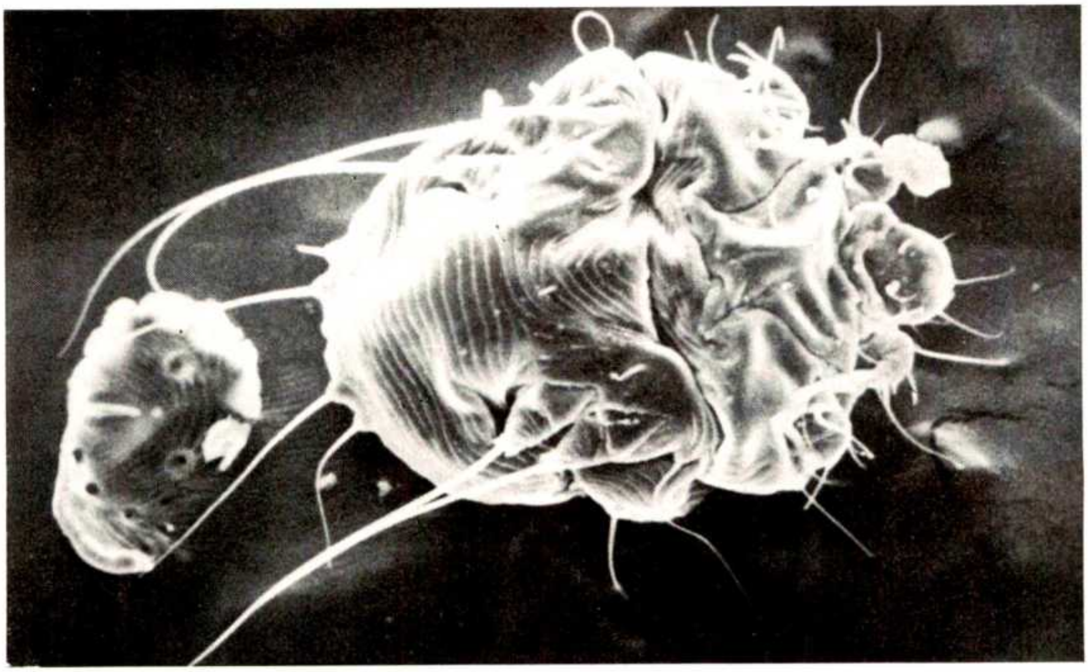

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Clinical and Laboratory Investigations

Mycosis fungoides—a disease of antigen persistence

R.S.-H.TAN,* C.M.BUTTERWORTH,† H.McLAUGHLIN,‡
SAMUEL MALKA† AND P.D.SAMMAN*

Departments of Dermatology and Chemical Pathology, Westminster Hospital, London

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SUMMARY

The function of B- and T-lymphocytes in nineteen patients with classical Alibert's mycosis fungoides (MF) was studied. In general there was no evidence of immune depression, not even in those with the disease for many years. The distribution of B-cells in the peripheral blood was normal. No immunoglobulin-bearing lymphocytes were detected in freshly liberated cells from the skin lesions or on direct immunofluorescence microscopy of those lesions. Serum IgE levels were significantly elevated, even though only two of the patients were atopic. 'Auto-antibodies' were detected in thirteen patients. Two cases of malignant lymphoma and one of Sézary syndrome contrasted strikingly with the mycosis fungoides (MF) group.

Our findings support the hypothesis that mycosis fungoides (MF) is a chronic granulomatous response to persistent unidentified antigen(s), upon which immune imbalance can develop resulting in 'auto-immune' phenomena and, in a few cases, the emergence of various lymphoreticular neoplasms.

The exact nature of mycosis fungoides (MF) as well as the clinical and histopathological criteria for its diagnosis are still in dispute. Most dermatologists regard it as being a reticulosis of the skin with a characteristic natural history and histopathology. However, evidence for it being a primary neoplastic condition is inconclusive and difficult to evaluate as different investigators have used different criteria for the disease and no convincing neoplastic cell has so far been demonstrated.

Recent work has shown that lymphocytes can be divided into two distinct types, those that have developed under the influence of the thymus—T-cells, and those that have been solely bone marrow derived—B-cells. Techniques are now available whereby these two types can be differentiated, depending upon the presence or absence of surface immunoglobulins (Raff, Sternberg & Taylor, 1970), or the presence of surface receptors to immunoglobulin, complement or sheep red cells (Basten *et al.*, 1972, Edelson *et al.*, 1973). These and other immunological techniques have been applied to the study of lymphoproliferative disorders such as lymphatic leukaemia (Aisenberg & Bloch, 1972, Ross *et al.*, 1973) and Hodgkin's disease (Gajl-Peczalska *et al.*, 1973).

We have studied B- and T-lymphocytes in the blood and skin of patients with MF. B-lymphocytes were identified by their surface-bound immunoglobulin using an immunofluorescent staining technique; their function was assessed by measuring serum immunoglobulin levels and by screening

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* Department of Dermatology, Westminster Hospital, London, S.W.1. † Department of Chemical Pathology, Westminster Hospital, London, S.W.1. ‡ Now at Department of Pathology, University College, Dublin 2.

for 'auto-antibodies' and Bence–Jones proteinuria. T-lymphocyte function was assessed by *in vitro* responses to phytohaemagglutinin (PHA), candida immunogen and a mixed lymphocyte reaction (MLR). These tests were made on freshly drawn blood taken on the same day and at the same time as that for the B-lymphocyte identification studies. In addition evidence of complement activation has been looked for.

TABLE 1. Clinical data

Patient number	Age	Sex	Diagnosis/stage (duration in years)	Total history in years	Histological diagnosis
1	59	M	Poikiloderma atrophicans vasculare	28	≡premycosis
2	54	M	MF plaques	23	≡reticulosis
3	57	M	MF plaques (1)	13	≡prereticulosis
4	57	F	MF plaques (8)	17	≡premycosis
5	62	M	MF plaques (5)	16	≡premycosis
6	33	M	MF plaques (3)	9	≡MF
7	66	M	MF plaques (1)+	2	≡MF
8	68	F	MF plaques (10)	15	≡MF
9	41	M	MF plaques (4)	8	≡MF
10	63	F	MF plaques (5) tumours (¾)	7	typical MF
11	37	M	MF plaques (8) tumours (3)	14	≡reticulosis
12	47	M	Premycosis (Alibert), poikiloderma	15	≡premycosis
13	60	M	MF plaques (4) tumours (1)	12	≡premycosis
14	65	F	MF epidermotrophic	9	epidermotrophic MF
15	69	F	MF tumours (3) ulceration (1)	40	MF
16	82	F	MF ulcerated tumours (½)	5	≡MF
17	70	F	MF plaques (½)	20	prereticulotic poikiloderma
18	53	M	MF plaques (¼)	13	MF
19	70	M	Premycosis (Alibert), poikiloderma	6	prereticulotic poikiloderma
20	67	M	MF plaques (1)+	3	MF
21	67	F	Sézary syndrome+*	2	active reticulosis
22	83	F	Lymphosarcoma*§	½	lymphosarcoma
23	62	F	Lymphocytic lymphoma+ ×	¼	malignant lymphocytic lymphoma

≡ compatible with; + normal bone marrow; × pathological fracture, bony deposits on skeletal survey; * 10–20% Sézary cells in peripheral blood and relative lymphocytosis; § relative lymphopenia (16% of 5800/mm^3).

PATIENTS AND METHODS

Twenty-three patients were studied (Table 1). They were selected from those attending Dr P. D. Samman's reticulosis clinic at St John's Hospital for Diseases of the Skin, or from those seen by him at Westminster Hospital. The diagnostic criteria were mainly clinical. The majority of cases were of the classical Alibert type of MF: an initial 'premycotic' stage, often of long duration, presenting as a non-specific patterned eczematous rash with variations in the redness of the patches, followed by the development of infiltrative skin plaques and progressing, in a minority, to frank tumour formation (Samman, 1970). In most of these the diagnosis was supported by the skin histopathology: a polymorphous infiltrate, often containing pleomorphic mononuclear cells, extending into the lower dermis or upwards into the epidermis with the formation of Pautrier micro-abscesses (Lever, 1967). In a few the histopathology was consistent with 'premycosis' or 'prereticulosis'. Three patients with other types of lymphomas affecting the skin were studied.

MF (Alibert). Nineteen patients were studied: twelve men and seven women, with a mean age of 58·9 years (range 33–82 years) and a mean total history of 13 years (range 2–40 years). Sixteen had infiltrative plaques; five of these had progressed to tumour formation. There was no clinical evidence of systemic involvement, and in the blood the total and differential white cell count were normal and no atypical cells were seen. Bone marrow examination of patients Nos. 7 and 20 was normal. The majority of patients had been on no specific therapy for at least 3 months; patient No. 11, however, had just finished a course of local superficial x-ray therapy 3 weeks previously and patients Nos. 10 and 15 were taking procarbazine and bleomycin respectively. There was one case of poikiloderma atrophicans vasculare (patient No. 1) which, after 28 years, had progressed neither to MF nor to a malignant lymphoma.

Sézary syndrome. Patient No. 21 presented with erythroderma and intense pruritus. Numerous Sézary cells were present in the peripheral blood. Lymph node histology showed benign hyperplasia, and bone marrow examination was normal. She had previously been treated with various cytotoxic agents and with the electron beam, but had discontinued all specific treatment for the preceding 2 months.

Malignant lymphoma. Two untreated cases were studied (patients Nos. 22 and 23). Both presented with rapidly developing skin tumours or nodules associated with erythematous patterning of the skin. Neither had significant lymphadenopathy or hepatosplenomegaly. In both, the bone marrow was normal and no atypical cells were found in the blood. Patient No. 22 died 3 weeks later. Patient No. 23 sustained a pathological fracture and subsequently had evidence of other bony deposits on skeletal survey. She died 5 months later.

B-Cell function

White blood cell and total lymphocyte counts were made on all patients.

Immunoglobulin-bearing cells—IgG-, IgA- and IgM-bearing cells in the peripheral blood were identified by the method as described by McLaughlin *et al.* (1973). Two hundred cells were counted for each sample, and the percentage of cells staining for the above surface immunoglobulins was determined. As the total lymphocyte count was known, the absolute numbers of each type of Ig-bearing cell could be calculated. Quality control normal samples were included in each batch.

Ig-bearing cells in the skin lesions were identified as follows: a skin biopsy was taken, half was placed immediately in Eagle's M.E.M. tissue culture fluid and washed. It was then replaced in fresh M.E.M., chopped finely with a scalpel to liberate the cells, and filtered through sterile gauze to remove the remnants of the tissue. The filtrate was centrifuged at 1500 rpm for 10 min, washed, and recentrifuged. The cells were finally resuspended in M.E.M. and treated as for the blood lymphocytes. The other half of the biopsy was snap frozen in liquid nitrogen; cryostat sections were cut and examined by direct immunofluorescence microscopy for immunoglobulins or complement.

Serum immunoglobulins—IgG, IgA and IgM were measured by a modified Mancini method (Hobbs, 1970) and IgE by radio-immuno-assay (Ezeoke, 1972).

Bence-Jones protein—concentrated urine samples were analyzed by cellulose-acetate electrophoresis (McLaughlin & Hobbs, 1973).

Tissue 'auto-antibodies' (Professor B. W. Lacey)—serum was screened for tissue 'auto-antibodies' by a standard indirect immunofluorescence technique on composite blocks of twenty tissues from different species. The presence of antinuclear antibodies was reported when they could be demonstrated in more than two of the tissues. The results were expressed as a reciprocal of the dilution (titre).

T-Cell function

Lymphocytes were harvested from defibrinated blood by sedimentation at 37°C in 3% gelatine in normal saline (Coulson & Chalmers, 1964). Lymphocyte transformation to phytohaemagglutinin (PHA) was assayed by the method of Yamamura (1973) and to candida immunogen by the method of Foroozanfer, Yamamura & Hobbs (1974). The mixed lymphocyte reaction (MLR) was tested using 2×10^5 lymphocytes from each patient cultured with an equal number of mitomycin C treated normal lymphocytes as stimulant, and incubating as for candida immunogen. All tests were set up in triplicate and a normal healthy control included in each experiment. The results were expressed as the transformation index (counts per minute stimulated/counts per minute unstimulated).

Serum complement levels were assayed by two-dimensional electrophoresis for C3 and C4, and for activation products C3i and C4i (Versey, 1973).

RESULTS

Serum immunoglobulin levels and the percentages of IgG-, IgA- and IgM-bearing cells in the blood and skin are given in Table 2. Serum IgG, IgA and IgM levels were not significantly different from normal although, in the nineteen MF patients, IgM was slightly depressed in three and raised in three, IgA was depressed in three and raised in one. However, the mean serum IgE level in the MF group was significantly elevated ($P < 0{\cdot}002$). Of the ten patients with raised IgE, only two (patients Nos. 9 and 11) could be considered as atopic. The two patients with malignant lymphoma and one with Sézary syndrome had normal immunoglobulin levels.

The distribution pattern of IgG-, IgA- and IgM-bearing lymphocytes in the peripheral blood was essentially normal in sixteen of the seventeen patients studied. Of the fourteen MF patients, five had a slight excess of IgG-bearing cells, but this is probably of no significance. The total numbers of cells bearing these three major immunoglobulin classes were mostly within our normal range. Patient No. 22, with malignant lymphoma did, however, have a greatly increased percentage of immunoglobulin-bearing cells in the peripheral blood.

TABLE 2. Serum immunoglobulin and distribution of immunoglobulin-bearing cells in the blood and skin

	Serum immunoglobulins				Immunoglobulin-bearing cells			
Patient number	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (Iu/ml)	IgG (%) in peripheral blood	IgA (%) in peripheral blood	IgM (%) in peripheral blood	(%) in skin lesion
1	11·0	2·90	1·40	300	ND	ND	ND	ND
2	11·0	3 50	0·39	275	10	6	3	few IgA
3	12·7	2·60	1 70	>4000	ND	ND	ND	0
4	10·0	1·50	2·10	363	ND	ND	ND	0
5	8 8	1·55	1·50	1000	11	8	4	0
6	7·0	0·84	1·40	1100	8	1	3	0
7	7·0	1·20	0 84	>4000	ND	ND	ND	0
8	15·2	3·10	1·25	150	21	10	1	ND
9	11 3	2·05	1·75	500	12	7	4	ND
10	6 7	0 98	0 33	180	11	0	6	ND
11	12 0	1·90	0·82	600	17	9	6	ND
12	12 1	1·60	0 69	500	33	12	9	ND
13	12 9	4·50	0·37	920	ND	ND	ND	ND
14	7·0	2 45	2·60	140	24	8	3	ND
15	11·8	2·65	0·54	1100	ND	ND	ND	ND
16	12·4	3·30	1·70	70	10	6	9	ND
17	7·4	6·80	0·80	720	11	4	5	ND
18	7 0	1 90	0 96	58	14	5	7	ND
19	8 8	3 00	1 10	10	20	5	0	ND
20	10·8	4 05	0·80	190	19	8	1	0
21	6·6	1·30	0·61	225	9	3	3	ND
22	9·0	1 50	0·66	300	50	20	7	10% IgM
23	8·3	3·00	1 50	26	18	5	0	ND
MF* Mean	9 8	2·28	0·97	368				
±2 S.D.	5·8–16·6	0·78–6·65	0·29–3·22	20–6841	9–17	3–10	0·5–8	Controls range
Control* Mean	9·5	2 50	0·95	105				
±2 S.D.	5·0–16·0	1·25–4 25	0 47–1·70	20–400				

* Log-normal ranges; ND not done.

The cells liberated from the 7 MF skin biopsies did not have detectable surface-bound immunoglobulin; but 10% of the cells from the patient with malignant lymphoma had surface-bound IgM. Neither immunoglobulin nor complement was detected in any of the eight cryostat sections examined by direct immunofluorescence.

At least one type of 'auto-antibody' was present in thirteen of the nineteen MF patients tested (Table 3). Antinuclear antibody was present, often to a high titre, in ten of these.

Bence–Jones protein was not detected in any of the fifteen urines analysed.

The results of the T-cell function tests are shown in Fig. 1. In the MF group, lymphocyte transformation was, in nearly every patient, within the normal range. Only patient No. 18 had depression of both the candida and MLR responses. However, in both cases of malignant lymphoma (patients

TABLE 3. Tissue 'auto-antibody' screen

Patient number	Antinuclear titre (pattern)	Tissue 'auto-antibody' titre
1	—	Gastric parietal cells 2560
2	10 (diffuse)	Thyroglobulin tanned cells 1000; thyroid colloid 10; microsomes 10; smooth muscle 10; axons 10
3	—	—
4	10 (diffuse)	Smooth muscle 10; striated muscle 10
5	—	—
6	—	—
7	320 (diffuse nucleolar)	Thyroid colloid 10
8	80 (diffuse)	—
9	—	Smooth muscle 10; axons 10
10	80 (diffuse)	—
11	—	—
12	—	Smooth muscle 10
13	10 (diffuse)	Elastin 10
14	10 (diffuse)	—
15	—	—
16	320 (diffuse)	—
17	20 (diffuse nucleolar)	Axons 10
18	—	—
19	—	Smooth muscle 10
20	80 (diffuse)	—
21	—	—
22	—	—
23	ND	ND

ND not done.

Nos. 22 and 23) all three tests were severely depressed. Patient 21 with the Sézary syndrome had depressed PHA and candida responses but a normal MLR.

C3i and C4i, indicative of immune complex formation, were found in two (patient No. 12 who had normal complement levels and patient No. 23 who had elevated C4).

DISCUSSION

Direct immunofluorescence studies, with fluorescein-conjugated sheep anti-human immunoglobulin, on cryostat sections of MF skin lesions and on cells freshly liberated from these lesions failed to show the presence of IgG-, IgA-, or IgM-bearing cells. The lymphocytes in the skin lesions of MF could therefore be other types of B-cells not identified in this study (IgD or IgE), T-cells, or immature cells (McLaughlin *et al.*, 1973). Edelson *et al.*, (1973) have suggested these cells to be T-cells. Broome *et al.*, (1973) and Winkelmann (1973) have proposed the same for the Sézary syndrome, considered by Clendenning, Brecher & Van Scott (1964), and Lutzner, Hobbs & Horvarth (1971) to be a form of MF. The distribution pattern of IgG-, IgA- and IgM-bearing cells in the peripheral blood was

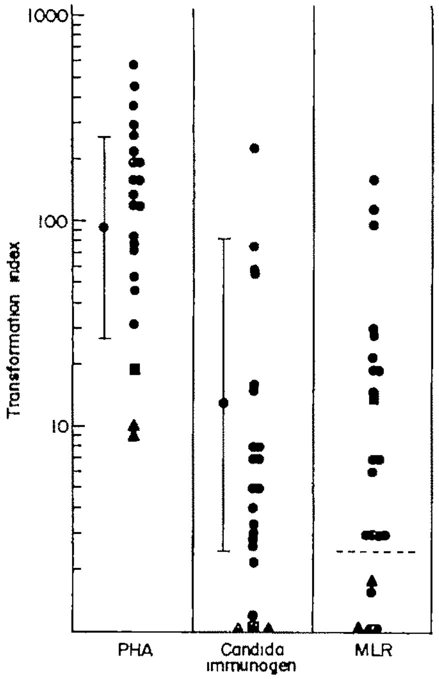

FIGURE 1. T-cell function: lymphocyte transformation to PHA, Candida immunogen and mixed lymphocyte reaction expressed as the transformation index. The bars represent the limits of normal range for PHA and Candida, and the horizontal dotted line the lower limit of normal for the MLR. ●, MF; ▲, Malignant lymphoma; ■, Sézary syndrome.

essentially normal in our MF patients, as were the total numbers of cells bearing these major immunoglobulin classes. Although these numbers do not represent the absolute numbers of circulating B-cells (IgD- and IgE-bearing cells were not identified) it is likely that they give a reflection of the total B-cell population. There was thus no evidence either of a gross excess or a deficit of circulating B-cells in our MF patients; neither was there evidence of an excess of circulating T-cells in these patients.

A patient with cutaneous lymphosarcoma, however, did have detectable immunoglobulin-bearing cells in the skin; she also had increased percentages of these cells in the peripheral blood. Recent work has shown that greatly increased numbers and percentages of circulating immunoglobulin-bearing cells are often found in chronic lymphatic leukaemia and in lymphosarcoma-cell leukaemia (Aisenberg, Bloch & Long, 1973). Our finding of essentially normal numbers and distribution of circulating B-cells in patients with MF (Alibert), taken in conjunction with the normal total white cell and differential counts, lack of atypical cells in the peripheral blood and the absence of any clinical evidence of visceral involvement in any of our cases, would indicate that this disease is confined to the skin with little tendency to disseminate. These features would be most unusual for a malignant neoplastic process.

We, as others previously, (Clendenning & Van Scott, 1965; Blaylock *et al.*, 1966, and Waldorf, Haynes & Van Scott, 1967) have found no evidence of immune suppression in the MF group. The lack of immunoglobulin deficiency many years after the onset of the disease would be against MF being a B-cell malignancy such as chronic lymphatic leukaemia (Aisenberg, Bloch & Long, 1973).

Similarly the normal *in vitro* T-cell tests are against MF being a malignancy resulting from a T-cell abnormality as has been postulated for Hodgkin's disease (Order & Hellman, 1972) in which impaired T-cell function is present early in the course of disease (Aisenberg 1966, Levy & Kaplan, 1974). Mild T-cell depression was present in only one MF patient (No. 18). This contrasts strikingly with the severe depression found in both patients with malignant lymphoma. These results emphasize the fundamental difference between the classical Alibert type of MF and the malignant reticuloses.

The so called 'mycosis cell' is now no longer considered specific to MF. Similar cells have been seen with the light microscope (Flaxman, Zelazny & Van Scott, 1971) and with the electron microscope (Lutzner *et al.*, 1971, and Flaxman *et al.*, 1971) in benign non-lymphomatous conditions. Vesper, Winkelmann & Hargraves (1971), using the skin window technique, considered they were benign primitive cells with no features of malignancy.

Bellanti & Green (1971) considered immunological reactivity as an expression of efficiency in the elimination of foreigness. If the foreign antigen persists, the immunological mechanisms of the body remain stimulated until eventually immunological imbalance results; the type and intensity of the host response becomes inappropriate leading to the most harmful sequelae of the immune reaction—those of auto-immunity or even malignancy. Immune imbalance causing auto-immune disease has also been postulated by Hobbs (1968). The concept of persistent antigenic stimulation would account for many of the features of MF. Thus, clinically and histopathologically a 'granulomatous' process is present; its behaviour is not neoplastic. Even the abnormal looking cells in the infiltrate may be benign primitive cells indicative of an intense cellular reaction by the host, or they may be T-cells reflecting an exaggerated response of the cell mediated type. IgG, IgA and IgM levels were essentially normal but IgE levels were significantly elevated and could be indicative of continued antigenic stimulation. However, at present, the significance of this finding is obscure. Further work on IgE levels is necessary with concomitant estimations of the percentage of IgE-bearing cells in the peripheral blood and in the skin lesions. Thirteen MF patients out of nineteen had evidence of 'auto-antibody' production, especially the anti-nuclear antibody which was present in ten patients. Ours was, however, a rather elderly group, and the association between 'auto-antibodies' and ageing is recognized. Even so the prevalence of tissue 'auto-antibodies' in our patients is in excess of that found by Hooper *et al.*, (1972) for a similar age group. We have been unable to demonstrate significant complement activation, but complement studies in the skin itself are needed before the role of complement in this disease can be dismissed, as local changes in the skin are unlikely to be reflected in the serum complement levels.

Undoubtedly a few cases of MF develop overt malignancy in the form of lymphosarcoma, reticulum cell sarcoma or Hodgkin's disease (Cyr, Geokas & Worsley, 1966). This multiplicity of malignancies that can arise would, in any case, be against a single cell neoplastic disorder as the cause of MF. It is more likely that one or other of the cellular elements of the infiltrate becomes malignant due to the continued antigenic stimulation (Burnet, 1964). Marshall (1956) suggested that the neoplastic change arising after an initial proliferative response could be due to an agent carcinogenic to one or other of the cellular elements of the reticular tissue. In this respect, the recent concept of Epstein & Achong (1973), in their study of Burkitt's lymphoma, of the ability of herpes virus to initiate non-productive and non-cytocidal infections which may subsequently undergo malignant transformation, is of particular interest. In view of the reported cases of malignant lymphomas, especially reticulum cell sarcoma, following kidney transplants (Penn, Halgrimson & Starzl, 1971), a situation in which persistent antigenic stimulation is combined with immunosuppressive therapy, it would seem hazardous to embark on intensive systemic antimitotic and anti-inflammatory therapy during the early stages of MF as neoplastic transformation may be hastened by the therapy.

Similarities between a graft-versus-host (GVH) reaction and MF have been stressed (Clendenning,

1971) and the GVH reaction and lymphomas have been discussed at length (Bierman, 1968). Although this concept would be consistent with many of the features of Hodgkin's disease (Kaplan & Smithers, 1959; Order & Hellman, 1972), such a reaction would seem unlikely in MF in view of the normal immunological status, lack of visceral lymphoid involvement and general well being of the patient. Walford (1966), however, has shown that GVH reactions can arise in mice without obvious runting and with no evidence of immunosuppression. Even so, the cellular infiltrate in the human skin in GVH is monomorphic with a predominance of lymphocytes and, in chronic cases, is associated with sclerosis of the papillary dermis (Kruger *et al.*, 1971). This is quite unlike the polymorphous picture in MF.

Research should now be directed into the nature of the initiating antigenic stimulus and the reason for the host's inability to eliminate it. The recent hypothesis of Ryan *et al.*, (1973) that MF can begin in the epidermis may be relevant and would be consistent with an exogenous factor in the pathogenesis of the disease. Emphasis should be placed on the fact that MF, at least initially, is a reactive granuloma rather than a malignant lymphoma. If the antigenic stimulus could be identified and eliminated early in the course of the disease, the subsequent immunological imbalance and neoplastic transformation would be unlikely to arise.

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The syndrome of chronic photosensitivity dermatitis and actinic reticuloid

W. FRAIN-BELL, T. LAKSHMIPATHI, J. ROGERS, AND JOYCE WILLOCK

Department of Dermatology, University of Dundee

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SUMMARY

In a study of thirty-four male subjects suffering from the syndrome of chronic photosensitivity dermatitis and actinic reticuloid the clinical, histological and photobiological features were such as to suggest that they were in fact examples of a single entity in which the degree of response to ultraviolet and visible light varied. Although a wide action spectrum involving UV and visible wavelengths invariably occurred with the classical clinical and histological features of actinic reticuloid (Ive *et al.*, 1969) a broad action spectrum with similar histological appearances was also noted in some of the subjects in whom the morphological changes were those of a chronic dermatitis confined to exposed sites.

We wish to report the clinical, histological and photobiological features observed in a group of thirty-four subjects affected by the chronic photosensitivity dermatitis and actinic reticuloid syndrome. In this condition the reaction of the skin to light may present as localized lichenified dermatitis of exposed sites or as the fully developed picture of actinic reticuloid (Ive *et al.*, 1969) in which there is involvement of exposed and covered skin along with lesions closely mimicking those seen in cutaneous lymphoma. Intermediate stages between these two extremes may occur.

CLINICAL FEATURES

The affected individuals are middle aged or elderly males who not infrequently have a past history of recurrent attacks of dermatitis of covered and/or exposed skin to which a constitutional (e.g. atopic, seborrhoeic) or idiopathic label has been attached, or in whom the dermatitis has been attributed to contact irritant or allergic factors sometimes with supporting evidence from positive patch test findings. The additional factor of an abnormal reaction to light may not appear for some years after the onset of the dermatitis although in some cases the interval may be only a matter of months. However, this influence of light on the skin may not be recognized by the patient.

As a result of this response to light, changes occur in the skin which may be either those of a chronic lichenified dermatitis of the head (i.e. face, scalp, neck and ears) and backs of the hands (Fig. 1) or

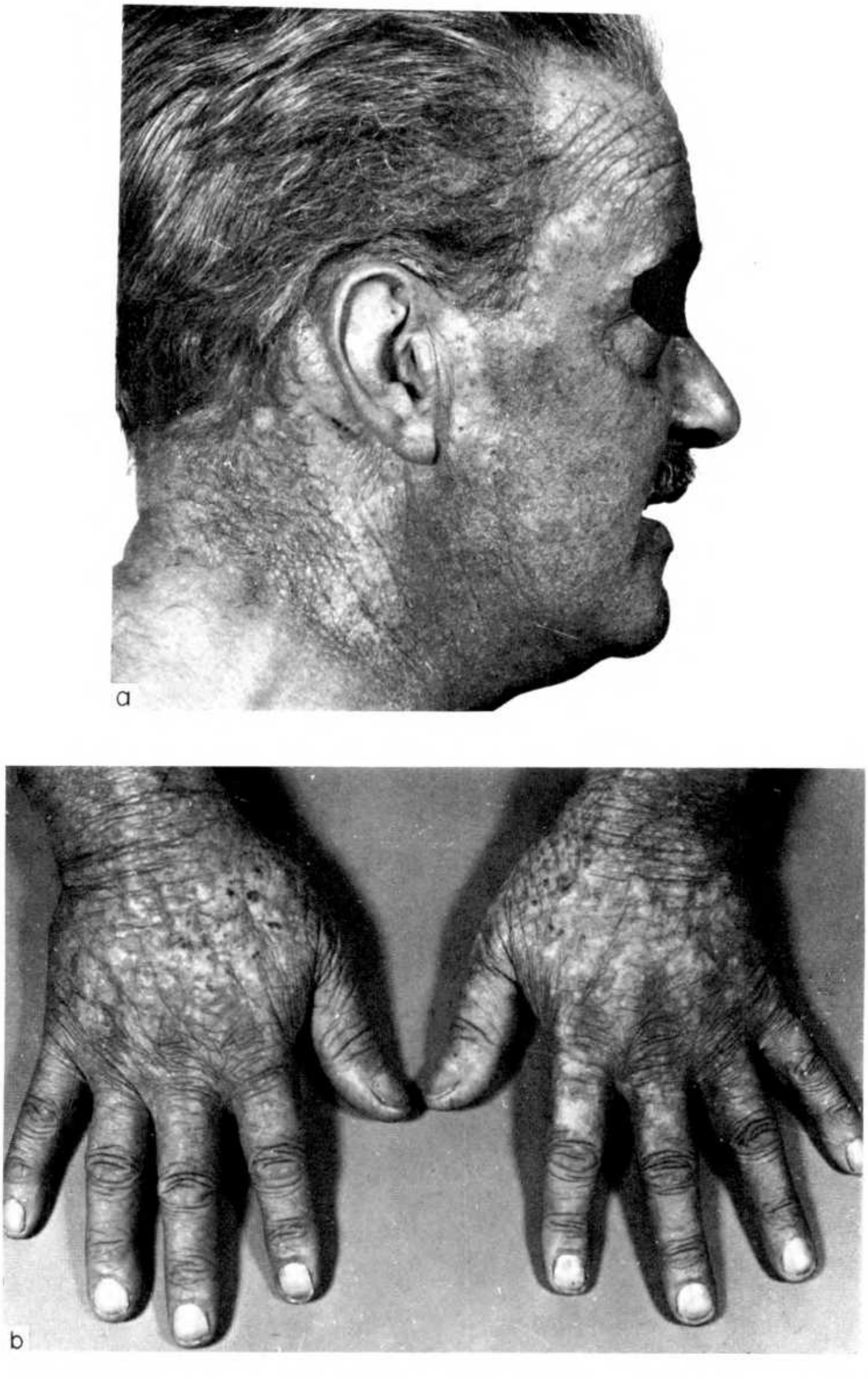

FIGURE 1a, b. Case No. 1, a forty-nine-year-old male showing lichenified dermatitis of the skin of the neck and backs of hands.

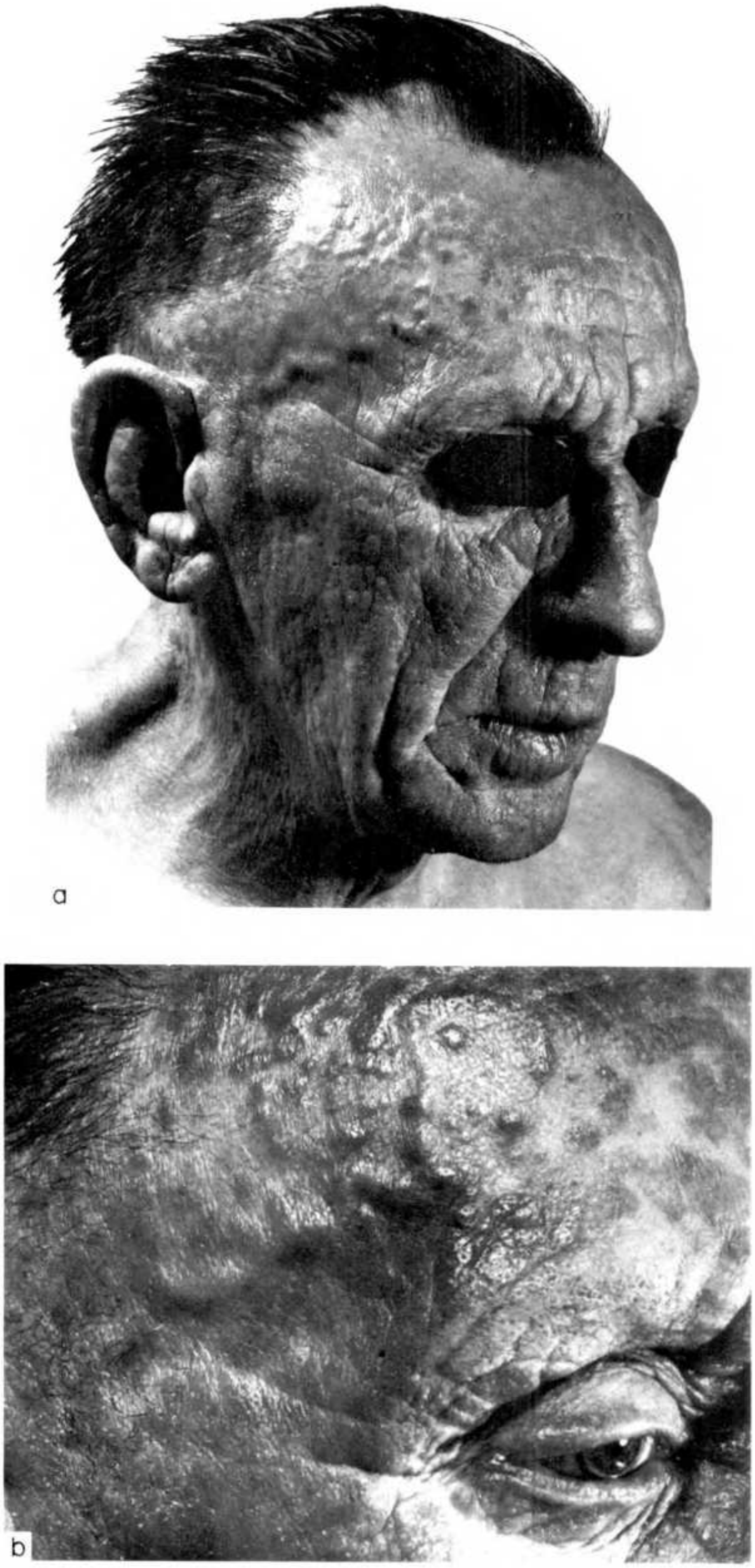

FIGURE 2a, b. Case No. 3, a sixty-seven-year-old male showing lymphoma-like skin change particularly noticeable over the right forehead.

lymphoma-like' lesions in the form of raised oedematous erythematous plaques and flat-topped variable sized papules affecting once again the head (fig. 2) and hands and frequently also covered parts of the trunk and limbs. Associated with this latter more severe reaction may be episodes of erythroderma which can spread widely over the body and may even on occasions become universal. In between these two extremes of response there are some individuals who will demonstrate the morphological changes of lichenified dermatitis interspersed with areas of lymphoma-like lesions or even of normal skin; although in these cases it is common for spread to take place to neighbouring parts of the shoulders, this mixed morphology of dermatitis and lymphoma-like change may be confined to the head and neck. A burning and prickling sensation may be complained of, but the main symptom is of itch which can be intolerable.

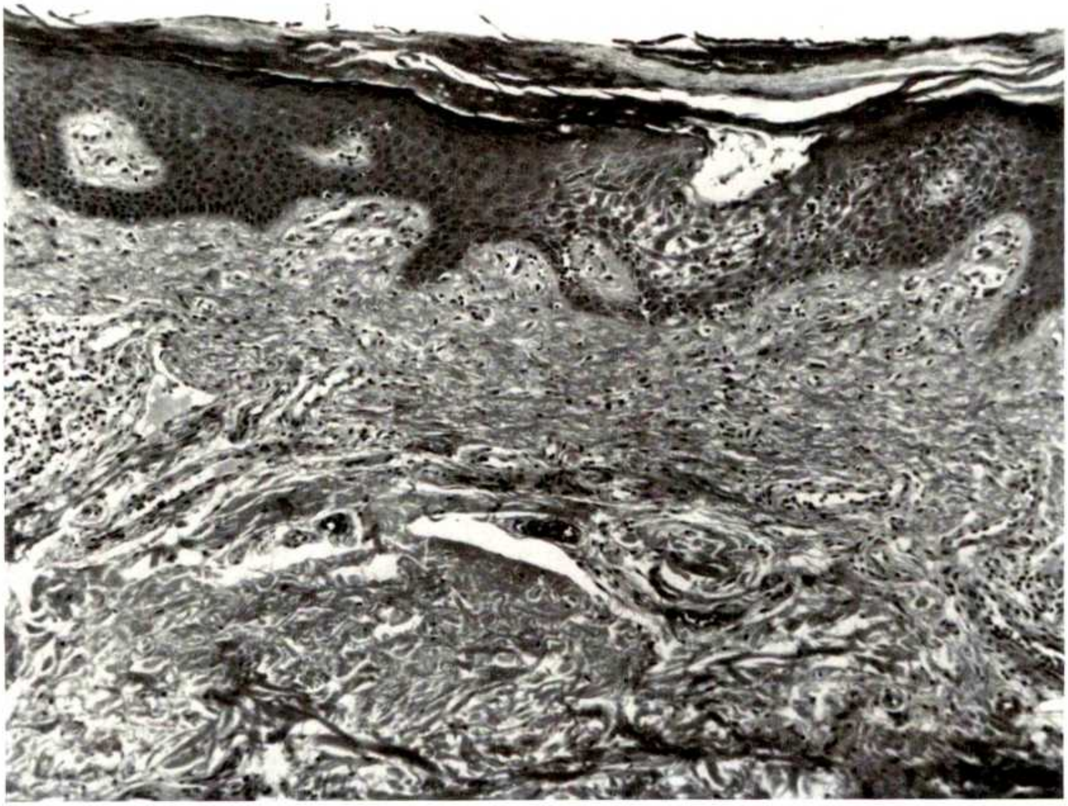

FIGURE 3. Case No. 18. Chronic photosensitivity dermatitis. Epidermis shows spongiosis, vesicle formation and parakeratosis. Peri-vascular lymphocytic infiltrate in dermis (H & E × 180).

A histological diagnosis of chronic dermatitis, rather than actinic reticuloid, is made when the epidermis shows mild to moderate eczematous changes in the form of inter- and intra-cellular oedema, at times progressing to vesicle formation and where patchy parakeratosis occurs following interruption of the normal maturation of the keratinocyte. In the dermis there is a perivascular infiltrate of lymphocytes and histiocytes confined to the superficial layers (Fig. 3).

In actinic reticuloid, the epidermis, despite invasion by inflammatory cells, shows little in the way of spongiosis and parakeratosis, and changes appear to be confined to a reactive acanthosis. In the dermis a dense polymorphic infiltrate extends deep around the adnexae and contains abnormal cells of the lymphocyte series with dark indented nuclei and occasional mitotic figures (Fig. 4a, b).

METHOD

Clinical, histological and photobiological features were recorded in thirty-four subjects with the chronic photosensitivity dermatitis and actinic reticuloid syndrome who had been referred for investigation during the period 1969–1973.

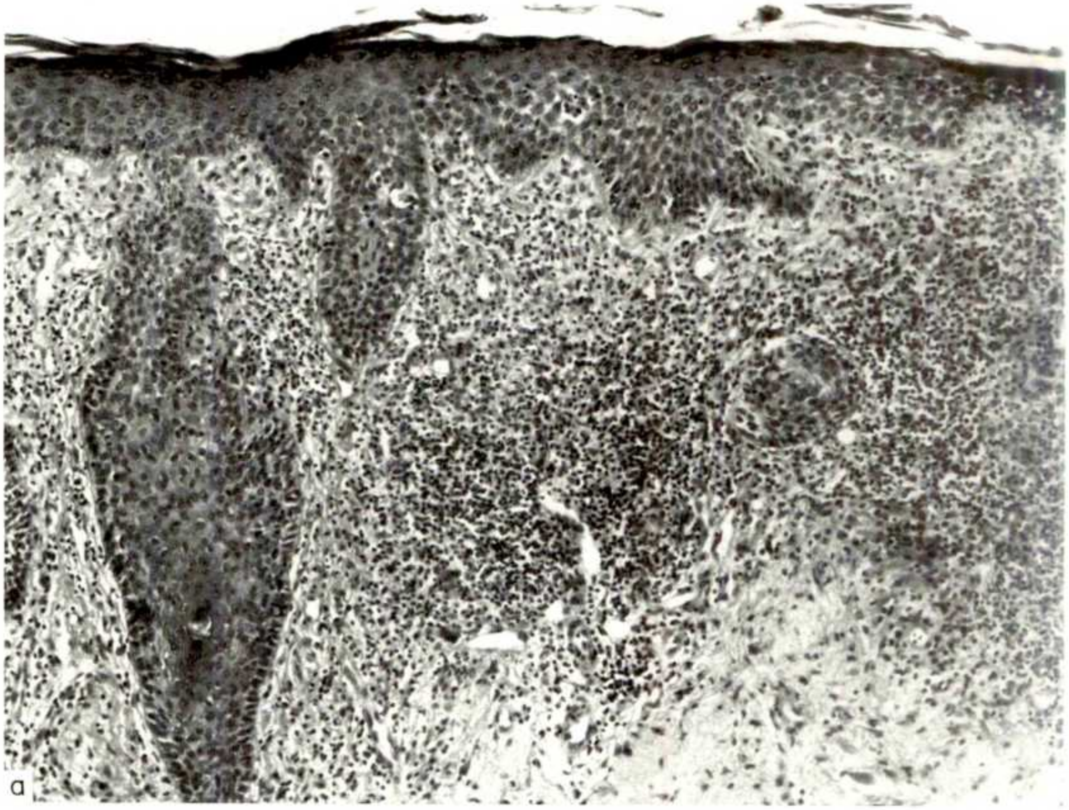

FIGURE 4a. Case No. 15. Actinic reticuloid. Epidermis infiltrated with inflammatory cells shows reactive acanthosis only. Dermal infiltrate dense and polymorphic extending deeply along adnexae (H & E × 180).

In addition to a routine physical examination along with certain haematological and biochemical tests (porphyrin estimations in blood, urine and faeces; serum proteins, urea and electrolytes, urinary amino acids, blood count and film, liver function tests) biopsies were taken from clinically affected skin in twenty-six cases and from artificially induced lesions at sites of irradiation on the skin of the back in twenty-two.

Action spectra for minimal erythema and/or abnormal papular responses were determined using a 1600 W xenon arc light source with a grating monochromator (Mackenzie & Frain-Bell, 1973).

The technique used has already been described (Frain-Bell *et al.*, 1973) and the wavelength and dosage range are shown in Table 1. Patch and photopatch tests to a series of chemical agents (tetrachlorosalicylanilide 0·5%, trichlorocarbanilide 1%, tribromosalicylanilide 1%, hexachlorophane 1%, all in an acetone vehicle; and Oil of Bergamot 10% in P.M.F.) were performed in thirty-two subjects, the light source being a black fluorescent tube (Philips Blacklight Tube TW20W/08, wavelength range 300–355–400 nm) placed at 10 cm from the patient's back, the irradiation time varying from 2 to 30 min depending on a previously defined minimal response dose for each subject. In this condition the severity of the response following irradiation of the skin of the back can be such that a full range of phototesting and photopatch testing is not always possible.

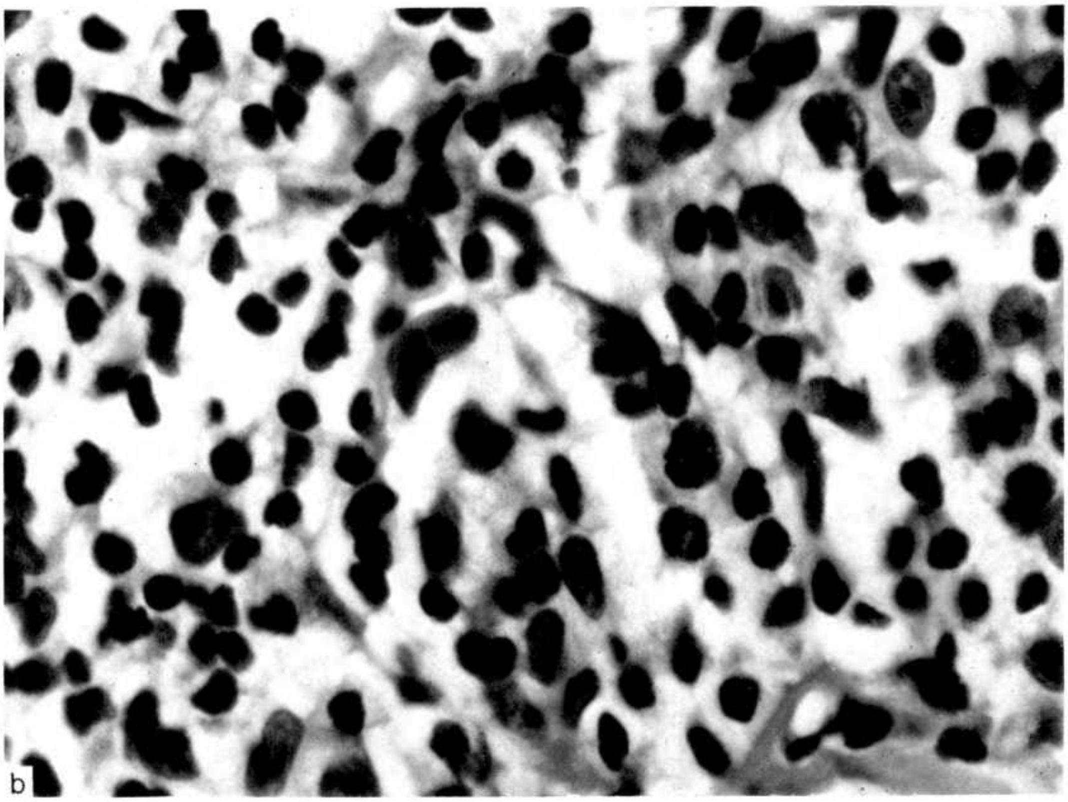

FIGURE 4b. Case No. 4. Actinic reticuloid. High power view of dense polymorphic infiltrate showing cells with hypochromatic, indented nuclei (H & E × 1100).

RESULTS

All thirty-four subjects were males ranging in age from the 30's to the late 70's with the majority, i.e. thirty-three (94%), over the age of 50 years (Table 2). A dermatitis of one kind or another had been present with variable severity and chronicity for less than 5 years in 40% to over 20 years in 23% (Table 3). The duration of the light sensitivity factor was less easy to determine and reliable figures were difficult to obtain. The abnormal skin changes were confined to exposed sites in nineteen subjects (56%), but covered sites were affected to a variable extent in 44%. Widespread erythro-

TABLE 1. Wavebands and dosage of irradiation used throughout the 3-day investigation

Day 1 Wavelength (nm)	Filter	Dose range
305–bandwidth (BW) = 290–320 nm	—	2·7–27 mJcm^{-2} in approx. 20% increment steps
305–BW = 300–310 nm	—	1·5–8·2 mJcm^{-2} in approx. 100% ,, ,, ,,
335–BW = 305–365 nm	WG 305 (2·5 mm)	100–10,000 mJcm^{-2} in ,, ,, ,, ,,
365–BW = 335–395 nm	WG 305 (2·5 mm)	560–22,000 mJcm^{-2} in ,, ,, ,, ,,
400–BW = 370–430 nm	WG 345 (2·5 mm)	22,000; 47,000 mJcm^{-2}
460–BW = 430–490 nm	GG 420 (2·5 mm)	82,000 mJcm^{-2}
500–BW = 470–530 nm	GG 475 (2·5 mm)	82,000 mJcm^{-2}
600–BW = 570–630 nm	OG 570 (2·5 mm)	82,000 mJcm^{-2}
DAY 2		
295–BW = 290–300 nm	—	0·5–10 mJcm^{-2} in approx. 20% increment steps
300–BW = 295–305 nm	—	0·5–27 mJcm^{-2} in ,, ,, ,, ,,
305–BW = 300–310 nm	—	5·6–68 mJcm^{-2} in ,, ,, ,, ,,
310–BW = 305–315 nm	—	22–68 mJcm^{-2} in ,, ,, ,, ,,
315–BW = 310–320 nm	—	47–470 mJcm^{-2} in ,, ,, ,, ,,
320–BW = 315–325 nm	—	330–1200 mJcm^{-2} in ,, ,, ,, ,,

Day 3

If 320 nm peak results were negative yet 365 and 400 nm peak results were positive and abnormal, the following irradiation was carried out:

Wavelength (nm)	Filter	Dose Range
320–BW = 310–330 nm	—	56, 120, 390 mJcm^{-2}
330–BW = 320–340 nm	WG 305 (2·5 mm)	220, 680, 1800, 3900 mJcm^{-2}
340–BW = 330–350 nm	WG 305 (2·5 mm)	680, 1800, 5600 mJcm^{-2}
360–BW = 350–370 nm	WG 305 (2·5 mm)	5600, 12,000 mJcm^{-2}
380–BW = 370–390 nm	WG 305 (2·5 mm)	5600, 12,000 mJcm^{-2}
400–BW = 390–410 nm	WG 375 (2·5 mm)	5600, 12,000 mJcm^{-2}

derma, whether fluctuating or persisting for long periods of time, occurred in only the comparatively small number of five subjects (15%) (Table 4).

The histological changes as defined on page 620 were those of actinic reticuloid in seventeen out of the twenty-six cases in which biopsies were carried out (i.e. in 69%) and those of a chronic dermatitis in eight (31%) (Table 5).

The action spectrum for abnormal morphological responses was confined to the short UV wavelengths (i.e. <320 nm) in only four cases (Nos. 17, 23, 24, 31). In nine subjects (Nos. 1, 2, 4, 9, 14, 22, 25, 26, 29) the long UV was involved as well and in the remaining twenty-one the action spectrum spread throughout the short and long UV and visible wavelengths. In these twenty-one with reactions to visible light, a 400 nm wavelength peak (bandwidth 370–430 nm) was involved in all instances; a 500 nm peak waveband (bandwidth 470–530) in eight (23%); and a 600 nm peak (bandwidth 570–630 nm) in three (8·5%). Thus in approximately 90% the action spectrum involved wavelengths beyond the short UV (Tables 6 and 7).

An attempt was made to correlate the clinical presentation with certain histological and photobiological features. Reference to Table 8 shows that no clear pattern emerged in that despite the reaction being confined to exposed sites in eighteen subjects, there appeared to be no restriction

TABLE 2. Sex and age distribution of the thirty-four patients

Case No.	Initials	Sex	Age
1	J.B.	M	49
2	W.McL.	M	34
3	G.C.	M	67
4	G.D.	M	52
5	D.McK.	M	63
6	J.L.	M	59
7	J.S.	M	65
8	F.C.	M	72
9	A.B.	M	61
10	D.H.	M	78
11	D.P.	M	63
12	B.C.	M	74
13	J.R.	M	60
14	D.B.	M	52
15	S.H.	M	72
16	D.S.	M	58
17	A.D.	M	54
18	A.A.	M	71
19	J.A.	M	74
20	J.McF.	M	53
21	M.E.	M	74
22	W.L.	M	57
23	J.T.	M	56
24	R.W.	M	71
25	N.N.	M	66
26	D.M.	M	68
27	D.McK.	M	67
28	J.D.	M	48
29	M.F.	M	53
30	R.McK	M	53
31	A.M.	M	63
32	J.E.	M	56
33	P.R.	M	74
34	D.L.	M	60

TABLE 3. Duration of dermatitis in the group of thirty-four patients

Duration of dermatitis (years)	<5	6–10	11–20	21–30	>30
No. of patients	14	9	3	4	4

TABLE 4. Cases in which either exposed sites only or exposed and covered areas were affected and in addition the few instances in which widespread erythroderma occurred

Case No.	Exposed sites only affected	Exposed and covered sites affected	Erythroderma
1	+		
2		+	
3		+	+
4		+	
5		+	
6		+	
7		+	
8	+		
9	+		
10	+		
11		+	+
12	+		
13	+		
14		+	+
15		+	+
16		+	+
17	+		
18	+		
19	+		
20	+		
21		+	
22	+		
23	+		
24	+		
25		+	
26	+		
27	+		
28		+	
29		+	
30	+		
31	+		
32	+		
33		+	
34	+		
Total	19 (56%)	15 (44%)	5 (15%)

TABLE 5. Histological features found in each case in either the clinical or induced lesion

Case No.	Clinical lesion	Induced lesion
1	AR	AR
2	AR	NS
3	AR	ND
4	AR	AR
5	DM	ND
6	ND	ND
7	AR	AR
8	DM	ND
9	DM	NS
10	AR	AR
11	AR	AR
12	AR	AR
13	ND	ND
14	DM	NS
15	AR	AR
16	AR	ND
17	DM	NS
18	AR	AR
19	AR	AR
20	AR	AR
21	DM	DM
22	AR	NS
23	ND	ND
24	DM	ND
25	DM	NS
26	ND	ND
27	ND	ND
28	ND	ND
29	ND	ND
30	AR	NS
31	ND	ND
32	AR	AR
33	AR	AR
34	ND	ND

AR, Actinic reticuloid; DM, Dermatitis; NS, Non-specific; ND, Biopsy not done.

TABLE 6. Wavelengths involved in the light sensitivity response in each case. Action spectrum

Case No.	290–320 nm	320–400 nm	400 nm (370–430 nm)	500 nm (470–530 nm)	600 nm (570–630 nm)
1	+	+	−	−	−
2	+	+	−	−	−
3	+	+	+	+	−
4	+	+	−	−	−
5	+	+	+	−	−
6	+	+	+	+	−
7	+	+	+	−	−
8	+	+	+	−	−
9	+	+	−	ND	ND
10	+	+	+	+	−
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+	+(430)	−	−
14	+	+	+	−	−
15	+	+	+	+	+
16	+	+	+	−	−
17	+	−	−	−	−
18	+	+	+	−	−
19	+	+	+	+	ND
20	+	+	+	−	−
21	+	+	+	−	−
22	+	+	−	−	−
23	+	−	−	−	−
24	+	−	−	−	−
25	+	+	−	−	−
26	+	+	−	−	−
27	+	+	+	−	−
28	+	+	+	−	−
29	+	+	−	−	−
30	+	+	+(430)	−	−
31	+	−	−	−	ND
32	+	+	+(460)	−	ND
33	+	+	+(430, 460)	−	−
34	+	+	−	−	−

ND, Irradiation omitted at this waveband.

The wavelength peaks in column 4, e.g. (430 nm) reference case No. 13, indicate that the wavelength peak was 430 nm (bandwidth 400–460 nm) and in case No. 32 460 nm peak (bandwidth 430–490 nm).

TABLE 7. Frequency of involvement of the various parts of ultraviolet and visible wavelengths in the total group of thirty-four patients

Wavelength (nm)	290–320	320–400	400 (370–430)	500 (470–530)	600 (570–630)
No. of patients	34	30	20	9	3

of action spectrum in comparison with that obtained when covered areas were affected as well (sixteen subjects); in fact, in each clinical group, i.e. ten out of eighteen and ten out of sixteen subjects respectively, there was an apparently similar spread into the visible wavelengths. Similarly, when the histological features of these two clinical groups were compared, the results were strikingly similar (Table 9) in that the features of actinic reticuloid or dermatitis were the same for each group, i.e. nine and four respectively.

TABLE 8. Relationship between the wavelengths involved and the site

	Action spectrum		
	Short UV	Long UV	Visible
Exposed skin affected only in 18 patients	18	14	10
Exposed and covered skin affected in 16 patients	16	15	10

TABLE 9. Relationship between the histological features of either dermatitis or actinic reticuloid and whether these were associated with affection of exposed sites only or exposed and covered areas

	Histological changes of dermatitis in 8 subjects	Histological changes of actinic reticuloid in 14 subjects
Subjects with exposed skin only affected	4	9
Subjects with exposed and covered skin affected	4	9

TABLE 10. Incidence of positive photopatch tests found in thirty-one of the thirty-four patients investigated and the number of positive responses obtained with each of the chemical agents used

Number tested—31 Number positive—10	Photopatch test results					
Substance	T_4CS	TBS	TCC	HEX	Bergamot	Others
Positive Results	9	3	3	4	2	2

T_4CS, Tetrachlorosalicylanilide; TBS, Tribromosalicylanilide; TCC, Trichlorocarbanilide; HEX, Hexachlorophane.

Despite the difficulty of performing photopatch tests and interpreting the results accurately on account of the reactivity of the skin in these patients, a significant number of positive responses were obtained and the interpretation of these findings will be referred to again. Suffice it to say that in one-third of the subjects tested a positive response was obtained to one or more photoactive chemical (Table 10).

DISCUSSION

In 1969 Ive, *et al.*, describing a group of ten patients, first drew attention to the clinical entitity of the middle aged and elderly male patient with photosensitivity in whom the morphological and histological features could be either those of a dermatitis or similar to those found in some of the cutaneous reticuloses. They chose to interpret this skin response as most probably that of an excessive reaction to light which was benign rather than that it represented evidence of irreversible malignant change, and they cited other examples of similar phenomena. Other cases have been reported (Heid *et al.*, 1970; Degos *et al.*, 1970; Degos, Civatte & Belaich, 1972), and metabolic abnormalities of the tryptophan–niacin pathway have been described (Binazzi & Calandra, 1969, 1971; Prandi *et al.*, 1972). More recently the suggestion that kynurenic acid may be a responsible factor has been made by Swanbeck & Wennersten (1973). There has been no report of the subsequent development of recognizable reticuloses although Jensen & Sneddon (1970) described a case of actinic reticuloid which was associated with reticulum cell sarcoma.

The relevance of phototoxic or photo-allergic responses to chemicals remains to be clarified although there is clinical and photobiological support for the view that photocontact dermatitis and the subsequent persistent light reaction are involved in some instances. The positive photopatch test reactions reported here in one-third of the cases suggest that photocontact factors are relevant and require further consideration. The increased reactivity of the skin on phototesting can make interpretation of the photopatch test results difficult and we propose to repeat this part of the study without prior phototesting so as to minimize the reactivity of the skin. Also, it was not appreciated that the small amount of short UV sunburn wavelengths contained in the black light emission could have produced erythema in some instances in the dosage used for irradiation of the photopatch tests. This probably did not produce false positive reactions in that the positive response was that appearing at the site of application of the chemical and recorded in comparison with any peripheral erythema occurring from the irradiation alone. However, currently photopatch testing is being done using a glass filter (Schott WG320, 2 mm thick) between the irradiation source and the subject's skin so as to remove all wavelengths shorter than 320 nm. Case No. 33 tested in this way gave a positive photopatch test to tetrachlorosalicylanilide and to hexachlorophane (see Table 11) suggesting that in fact the technique probably was not responsible for false positive reactions although the reactivity of the skin may well have been in some instances. We hope to report later the results of further photopatch test studies in these subjects.

The action spectra results referred to on pages 623 were based in most instances, unless prevented by the excessive reaction of the skin, on a combination of initial broad waveband and subsequent narrow waveband irradiation throughout the short and long UV (Table 1) giving the type of classical action spectrum as seen in Fig. 5a, b, c. It is interesting also that in some subjects a broadening of the action spectrum on repeat phototesting was found. This apparent ability to react to an increasing span of light is being further assessed.

Although we have chosen to define histologically those cases in which the appearances were those of actinic reticuloid (AR) in comparison with those found in dermatitis this was not always an easy and clear-cut decision.

The majority of cases having a typical history and morphology of AR showed the deep and dense infiltrate simulating lymphoma but some with a similar history and appearance had to be classified as dermatitis. Presumably the histological picture is influenced by a number of factors such as recent exposure to light or the recent use of local or systemic corticosteroid drugs. It was noted that when the initial biopsy was taken from a site where gross basophilic degeneration was present the infiltrate was less dense than expected and was limited to the area between the epidermis and the masses of

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TABLE 11. Individual cases in which positive photopatch test results were obtained and which of the chemicals were responsible

	Photopatch test results					
Case No.	T_4CS	TBS	TCC	HEX	Bergamot	Others
5	+	+	+	−	−	
8	+	−	+	−	+	
9	+	+	+	−	−	
14	−	−	−	−	+	
18	+	−	−	−	−	+(TAR)
21	+	+	−	−	−	
22	+	−	−	+	−	
24	+	−	−	+	−	+(Irgasan)
27	+	−	−	+	−	
33	+	−	−	+	−	

T_4CS, Tetrachlorosalicylanilide; TBS, Tribromosalicylanilide; TCC, Trichlorocarbanilide; HEX, Hexachlorophane; TAR, Coal Tar BP 0·1% in Industrial Spirit; Irgasan, DP.300 (Tricloro 2,4, 4^1-trichloro-2^1-hydroxydiphenyl ether.

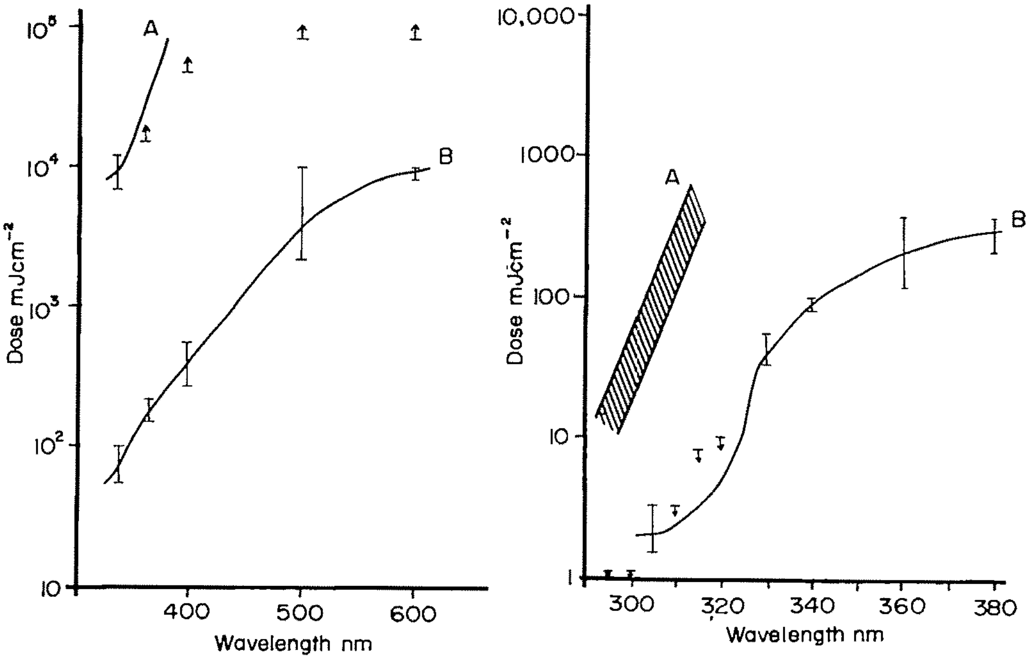

FIGURE 5a. The type of action spectrum commonly obtained in this group of patients in which there is involvement of UV wavelengths both short and long and also into the visible light as far as 600 nm peak (Case No. 12). In both graphs (5a, b) B refers to the action spectrum of the patient, and A to the action spectrum for the same wavelengths obtained with a group of nineteen normal subjects. In A the bar refers to the mean and one standard deviation from the mean. The line A (5a) was drawn from the single defined minimal erythema dose level within the line of the arrows denoting that a minimal erythema dose level at these two wavelengths had not been reached. In B the bar refers to the mean and one standard error from the mean. ↑In both graphs (5a, b) this sign denotes that the minimal erythema dose was not reached, the maximum dose applied at these wavelengths being represented by the base line of the arrow. ↓Where the arrow points downwards, the no response dose level had not been reached.

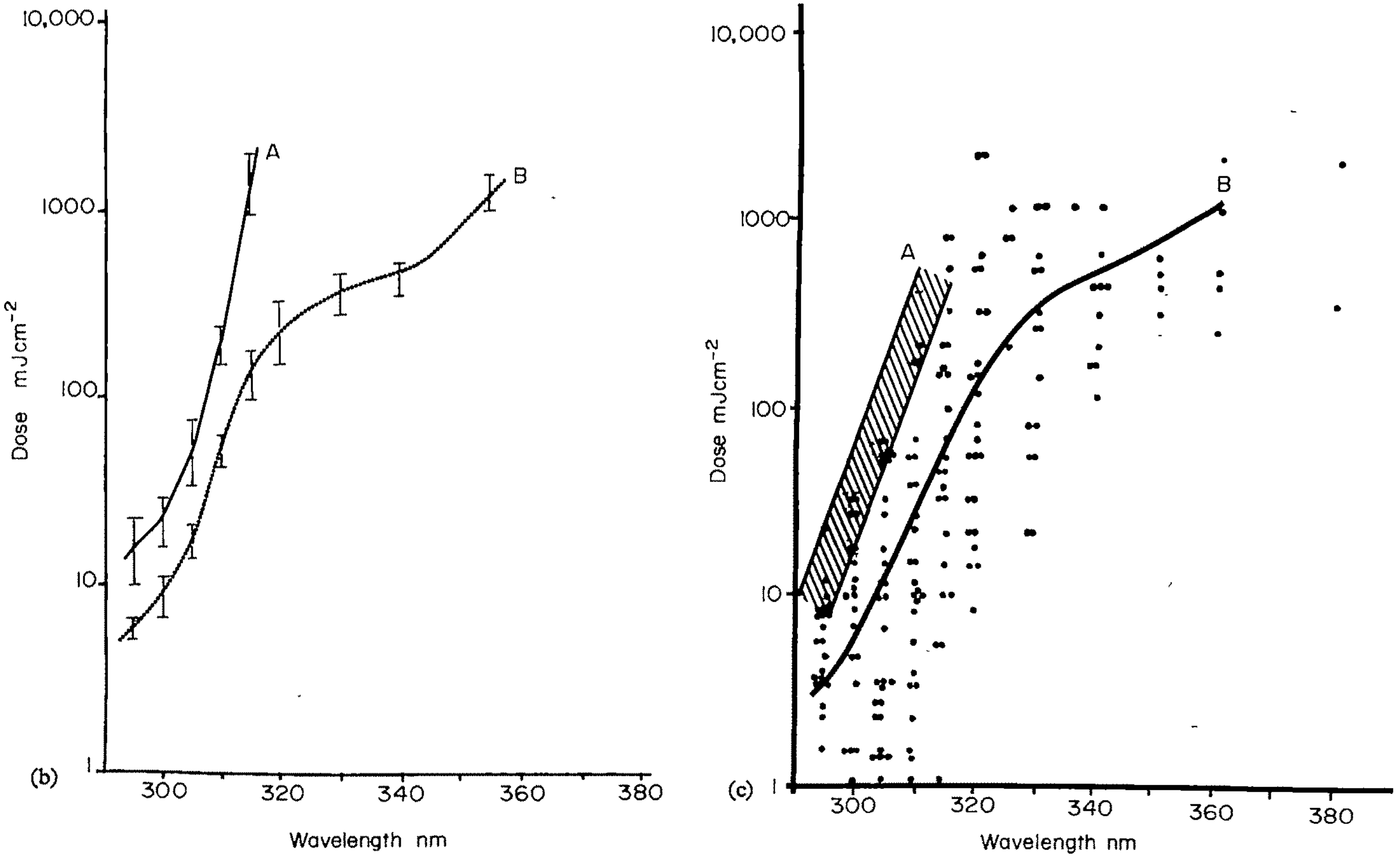

FIGURE 5b, c. The spread of the action spectrum in the ultraviolet for the whole group of thirty-four patients, once again A referring to normal subjects and B to those with chronic photosensitivity dermatitis/actinic reticuloid. The single dots in 5c refer to the minimal abnormal response dose for a particular subject. In A the bar refers to the mean and one standard deviation from the mean and in B to the mean and one standard error from the mean.

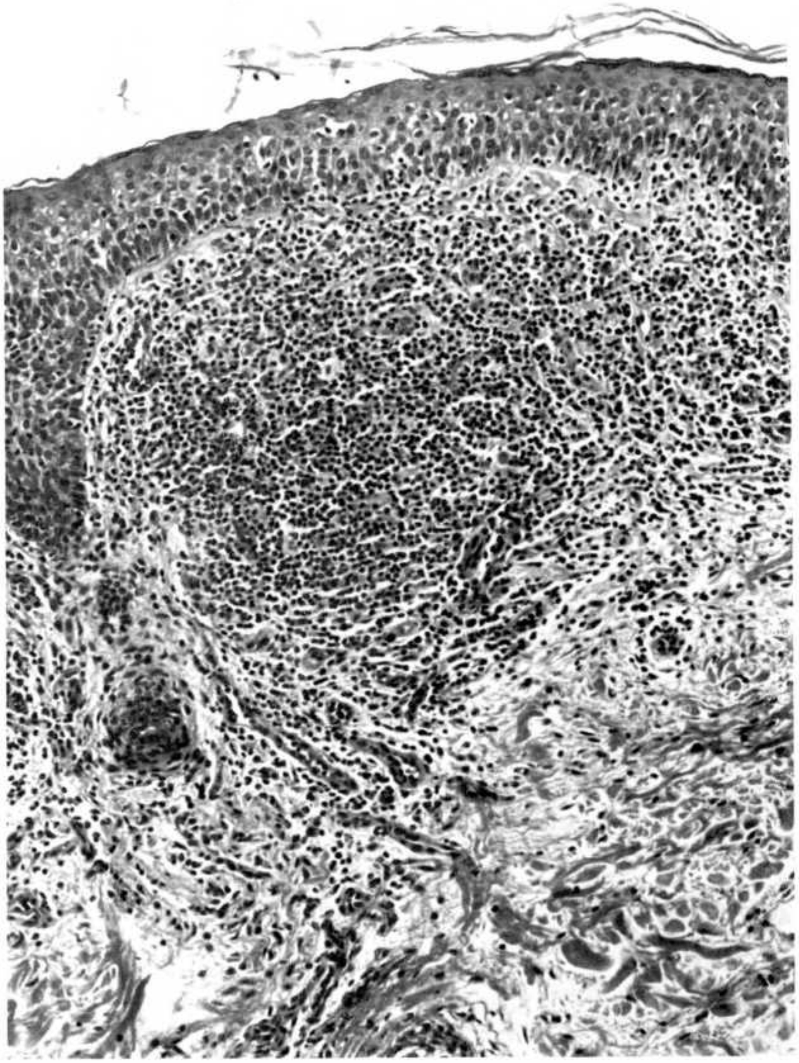

FIGURE 6. Case No. 15. Induced lesion in actinic reticuloid, the biopsy being carried out 24h after irradiation at wavelength 400 nm peak (bandwidth 370–430 nm) with a dose of 16,000 mJcm $^{-2}$.

TABLE 12. Incidence of the histological features of either AR or chronic dermatitis in the biopsy material obtained from the clinical reaction or induced reaction sites

			Clinical reaction in 25 subjects	Induced reaction in 22 subjects
Histological changes of	Actinic reticuloid	in	17	12
	Dermatitis	in	8	1
	Non-specific	in	0	9
	Not done	in	9	12

altered collagen. In early studies it was thought that the tissue eosinophilia which occurred as part of the polymorphic infiltrate might be useful in differentiating the AR cases from those of chronic dermatitis but this feature has been noted in both. Nevertheless, the spread of eosinophils into the epidermis between essentially normal basal cells was seen only in the AR cases. It is probable that the reaction of the epidermis to the infiltrating inflammatory cells is of importance in differentiating the AR type of response from that seen in chronic photosensitivity dermatitis in that the large number of cells which invade the epidermis in the AR form induce only a reactive acanthosis; whereas in the dermatitis form fewer invading cells are accompanied by gross spongiosis progressing to vesicle formation and marked parakeratosis. This feature is even more marked in biopsy tissue taken from induced lesions in cases with a clinical presentation of AR where a dense infiltrate of cells in the upper dermis can be seen invading an epidermis which shows little in the way of eczematous changes (Fig. 6); lymphocytic cells are in fact streaming into the epidermis only 2 h after irradiation. When the clinical picture is more that of a chronic dermatitis, biopsy material from the induced lesion often shows only a mild perivascular infiltrate in the upper dermis with little change in the epidermis, except in more acute reactions to irradiation when eczematous changes are also present.

Examination of the dermal response in the induced lesions in the AR cases showed the polymorphic infiltrate extending deeply along the adnexae. Large atypical cells could be identified with relative ease and the number of cells in mitosis appears to relate to the density of the infiltrate.

Collections of cells in the epidermis simulating Pautrier abscesses were seen in only two cases (4 and 10) and in the clinical lesions only.

The histological features seen in both clinical and induced lesions are summarized in Table 12.

There is little doubt therefore that certain middle aged and elderly men are prone to develop dermatitis, in which a light sensitivity factor is present, involving wavelengths which vary in distribution throughout the ultraviolet and visible spectrum. In these subjects the clinical appearance of the skin reaction may be that of a dermatitis or of a cutaneous reticulosis, or a mixture of both in a distribution which may be restricted to exposed sites or spread to covered areas. The histological features may be either those seen in dermatitis or more those of a malignant reticulosis. The action spectrum will in the majority of subjects involve both the UV and visible wavelengths, but may be restricted to short and long UV with perhaps the potential for extension with the passage of time.

The controversial issue is whether there are in fact two entities, i.e. photosensitivity dermatitis on the one hand and actinic reticuloid on the other. Ramsay & Black (1973) have recently described a group of male patients usually with long-standing eczema in whom light sensitivity has subsequently developed and in whom the responsible action spectrum is confined to the sunburn ultraviolet radiation (290–320 nm wavelength). They have called this condition Photosensitive Eczema and separated it from actinic reticuloid although mentioning that some of the clinical features are common to both conditions. We believe, however, that the absence of any close correlation between either clinical presentation and clear-cut differences in the photobiological and histological features point towards a single entity. It would seem reasonable therefore to include this as a single condition within the idiopathic photodermatoses along with polymorphic light eruption and summer prurigo with the proviso that further study of photocontact factors is indicated.

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Pathogenesis of diffuse melanosis secondary to malignant melanoma*

K. KONRAD AND K. WOLFF

Division of Experimental Dermatology, Department of Dermatology I., University of Vienna,
Alserstrasse 4, A-1097 Vienna, Austria

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SUMMARY

The heavily pigmented but otherwise normal appearing skin of a patient with diffuse melanosis secondary to metastatic malignant melanoma was examined by electron microscopy and electron microscopic cytochemistry. The findings indicate that the slate-blue colour of this melanosis is due, for the most part, to melanosomes derived from melanoma cells that have spread to and throughout the skin of the patient. The dissemination of individual, discrete tumour cells within the patient's dermis represents a unique form of single cell metastasization.

The diffuse, slate-blue melanosis in patients with advanced metastatic melanoma has been attributed to an oxidation of circulating melanin precursors into melanin within the extracellular fluid or within histiocytes of the patient's dermis (Fitzpatrick, Montgomery & Lerner, 1954); or to the deposition of melanosomes, derived from distant melanoma cells, that are carried to the skin by the blood stream (Silberberg, Kopf & Gumport, 1968). In this paper we present evidence that a different mechanism can be operative in the pathogenesis of this universal pigmentation: an unlimited spread of individual, discrete melanoma cells to and throughout the entire skin.

CASE REPORT

A 58-year-old man was admitted to hospital in May 1971 with a pigmented lesion that had arisen from a mole and, within 5 months, had doubled in size. On several occasions bleeding had occurred after slight trauma.

On examination the patient was in good general health. His eyes were pale blue and his grey hair showed traces of red. Upon questioning he indicated that in his youth he had had red hair. His skin was fair and freckled and was studded with multiple small lentigines. On the left side of the anterior chest, 5 cm above the costal margin, there was a flat, deeply pigmented nodular melanoma measuring 2 cm in diameter; two pea-sized lymph nodes were palpable in the left axilla. Physical and laboratory examinations revealed no evidence of distant metastases; the urine was normal in colour and the chemical tests for melanin precursors were negative.

The tumour was excised with ample margins and the histological examination revealed a malignant melanoma. Five days after this operation the tissue surrounding the primary site was resected and a radical lymph node dissection of the left axilla followed by split skin grafting was performed. Histo-

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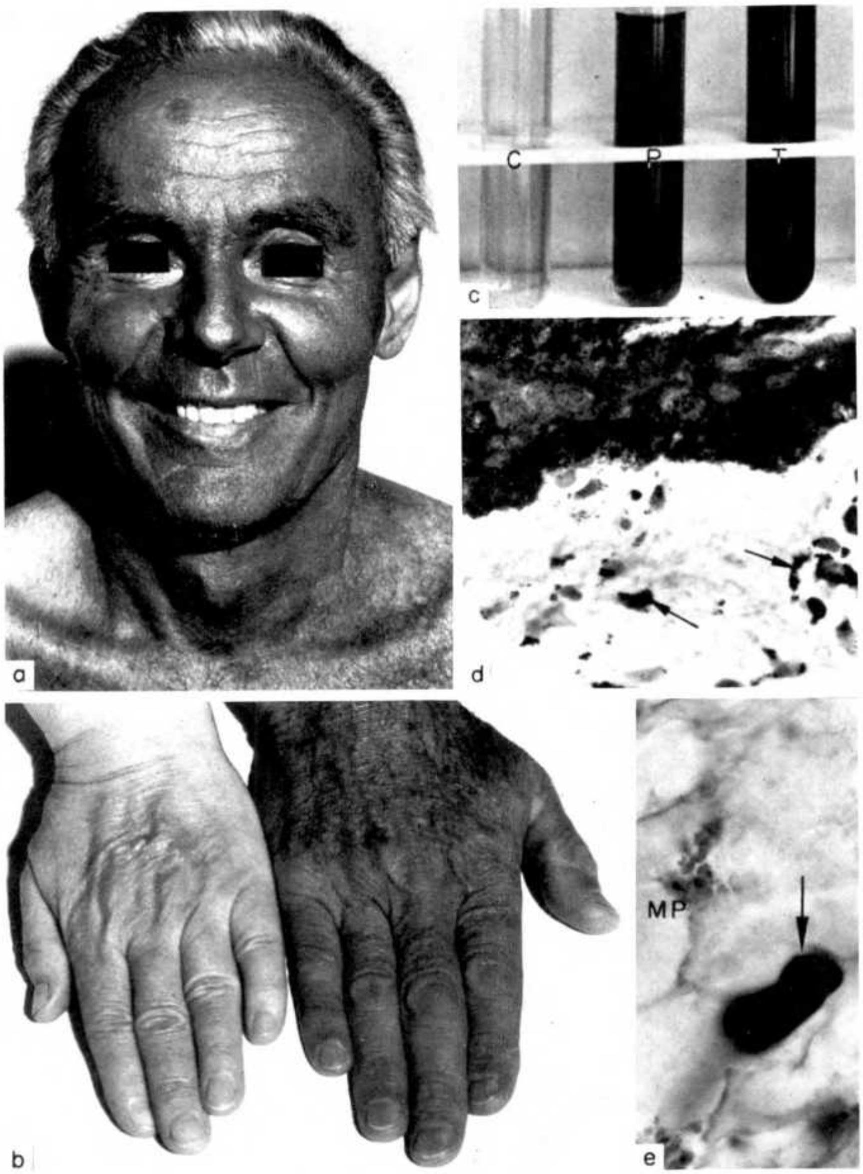

FIGURE 1a. Clinical appearance of the patient; 1b. The patient's hand compared with that of a control person. Note pigmentation of the nail beds; 1c. Urine of the patient after exposure to light and air (P) and after the Thormaehlen test (T); C control; 1d. The histology of normal appearing skin shows a moderate number of dermal melanin-bearing cells around small blood vessels and dispersed diffusely between the collagen bundles (arrows). There is hyperpigmentation of all layers of the epidermis. Masson's silver method (× 240); 1e. A strongly DOPA-positive dermal cell (arrow) as seen at the light microscope level. MP indicates melanophage from which reaction product is absent (× 1100).

logically, several foci of melanoma cells were found in one of the lymph nodes and also in the surrounding adipose tissue. Telecobalt irradiation of the supra- and infraclavicular regions was performed after surgery.

After discharge the patient was in good health and remained free of disease until December 1971 when several lenticular nodules of slate blue colour appeared within and around the grafted area. One of these was excised and was found to be metastatic melanoma. Telecobalt irradiation led to a local regression of the secondary nodules but in January 1972 there was a rapid and widespread dissemination of multiple skin metastases. Telecobalt, strontium and X-ray irradiation was given to multiple isolated lesions. Four months later the patient noted a greyish discolouration of his skin and the conjunctival mucous membranes. The pigmentation was steadily progressive and within a few months resulted in a uniform, slate-blue melanosis. The patient remained in a remarkably good state of health but in October 1972 he began to lose weight and his condition deteriorated rapidly. When he was readmitted to hospital his entire skin and all visible mucous membranes had a gunmetal-like colour, the pigmentation being most pronounced on the hands, on the arms, shoulders, upper chest, neck, and face (Fig. 1a). The nail beds also exhibited a bluish discolouration (Fig. 1b). There was an admixture of brown with the slate-blue colour in the light exposed regions, and on the shoulders the pigmentation was so intense that the skin appeared almost black.

Multiple flat nodules measuring from a few millimetres to 2 cm in diameter were seen and palpated on the trunk and the extremities; most of them were pitch-black. The oral and pharyngeal mucous membranes were also diffusely pigmented and several black nodules were present on the soft palate, at the base of the tongue, in the left tonsillar fossa, and in the pharynx.

Apart from a raised sedimentation rate and scintigraphic evidence of liver metastases all laboratory and X-ray examinations yielded unremarkable results. The urine exhibited a brown colour when passed and darkened still further upon standing. The chemical reactions for melanin precursors were positive (Fig. 1c).

The patient's condition deteriorated further and he died 3 weeks after admission due to cardiac failure. Up to his death there was a steadily progressive increase of his melanotic pigmentation.

Autopsy revealed a diffuse grey pigmentation of the pharyngeal, tracheal and oesophageal mucous membranes; of the intima of the aorta and pulmonary artery; and of the renal pelvis and the ureters. The liver, spleen, kidneys and bone marrow exhibited a diffuse or mottled grey to black discolouration and so did the heart. Innumerable nodular metastases of pitch-black colour were found in the skin, in the skeletal muscles and subserous fatty tissues; nodular metastases measuring from a few millimeters to 4 cm in diameter were present in all internal organs and were particularly numerous in the heart, liver, spleen, pancreas, suprarenals, and the gastrointestinal tract; the mediastinal, para-aortic, and inguinal lymph-nodes were partly or completely replaced by pigmented tumour.

INVESTIGATIONS

Special investigations were performed on deeply pigmented skin obtained by shave biopsy from the patient's trunk 3 weeks prior to his death. This skin showed no evidence of metastases and, apart from its bluish-black colour, appeared macroscopically normal.

METHODS

Light microscopy

Specimens were embedded in paraffin for routine histology and sections were stained with haematoxylin and eosin and Masson's silver method. Darkfield examinations were performed to exclude the presence of silver as a possible cause of the slate-blue pigmentation (Hönigsmann, Konrad & Wolff, 1973). For histochemical purposes, the specimens were snap frozen in dry ice and cut on a cryostat. Sections were stained with a modified DOPA method (Becker, Praver & Thatcher, 1935) to reveal tyrosinase activity and were incubated according to Grogg & Pearse (1952) to demonstrate acid-phosphatase.

Electron microscopy

Strips of skin measuring 1 × 1 × 5 mm were fixed in a 1:1 dilution of Karnovsky's (1965) paraformaldehyde-glutaraldehyde fixative for 3 h at room temperature. After an overnight rinse in 0·1 M cacodylate buffer they were either subjected to osmification or processed for the ultrastructural demonstration of tyrosinase and acid-phosphatase according to procedures routinely used in our laboratory (Konrad, Wolff & Hönigsmann, 1974a). The specimens were embedded in Epon; Reichert OM U2 and LKB ultrotome III ultramicrotomes were used to cut ultrathin sections; a Zeiss EM 9S electron microscope was employed in the ultrastructural studies.

RESULTS

Light microscopy

At low magnifications the skin appeared structurally normal and, apart from the pigmentary changes to be described, there were no major pathological alterations.

Foci of pigmentation in the lower papillary and upper reticular dermis were the most conspicuous finding (Fig. 1d). This pigment was confined to large (presumably) histiocytic cells and was shown, by silver impregnation, to represent melanin. The pigment-bearing cells were clustered around small blood vessels but were also found dispersed, in a haphazard fashion, distant from vascular channels. Many of these cells exhibited acid-phosphatase activity which confirmed that they represented macrophages. However, in cryostat sections incubated with DOPA it was noted that a considerable number of these cells, particularly the larger ones, were strongly DOPA-positive (Fig. 1e).

Careful screening of semithin sections eventually disclosed that the pigment bearing dermal cell population consisted of two cell types. The majority represented macrophages containing large amounts of aggregated melanin. The second cell type was much larger in size and differed from the melanophages by an even distribution of cytoplasmic melanin granules and by the fact that they often contained multiple nuclear profiles and clear cytoplasmic vacuoles (Fig. 10, inset). These cells corresponded to the DOPA-positive cells described above and, as will be described below, were identified as melanoma cells at the ultrastructural level.

In a few instances two or three melanoma cells were aggregated to form small clusters that were surrounded by a few lymphocytes. In general, however, inflammatory cells and lymphocytes were sparse. A careful search revealed melanoma cells even within small dermal blood vessels (Fig. 4, inset). The endothelial cells of the dermal capillaries frequently contained coarse aggregates of melanin granules.

The epidermis was strongly hyperpigmented as was particularly evident in sections stained with the silver method. Melanin granules were densely aggregated in basal and suprabasal cells but also extended into the stratum corneum (Fig. 1d). The basal melanocytes were DOPA-positive but were not increased in number. Semithin sections cut from Epon blocks revealed that many melanocytes were vacuolated (Fig. 12, inset). Occasionally, large non-keratinocytes were seen within the epidermis that were two or three times larger than any normal epidermal cell (Fig. 13, inset). They were irregular in outline and some were multinucleated. Their cytoplasm was clear, contained vacuoles, and appeared

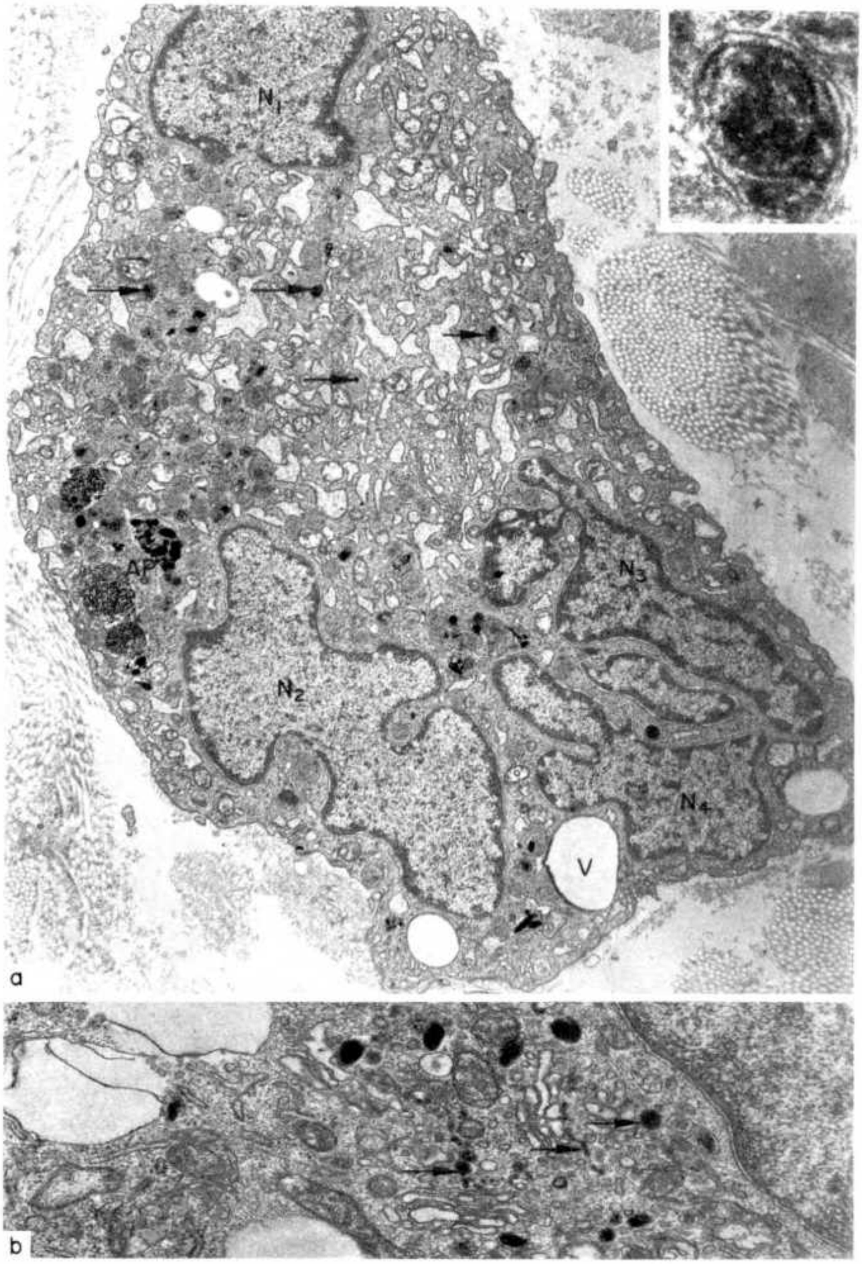

FIGURE 2a. Electron microscopic appearance of an individual melanoma cell between the fibrous components of the dermis. The arrows mark several melanosomes dispersed in the cytoplasm; N$_{1-4}$ several nuclear profiles; AP, autophagosomes; V, vacuoles (× 7700). Inset: abnormal melanosome at high magnification (× 66,900); 2b. DOPA reaction product (arrows) in the Golgi area and in pre-melanosomes of a melanoma cell within the dermis (× 17,800).

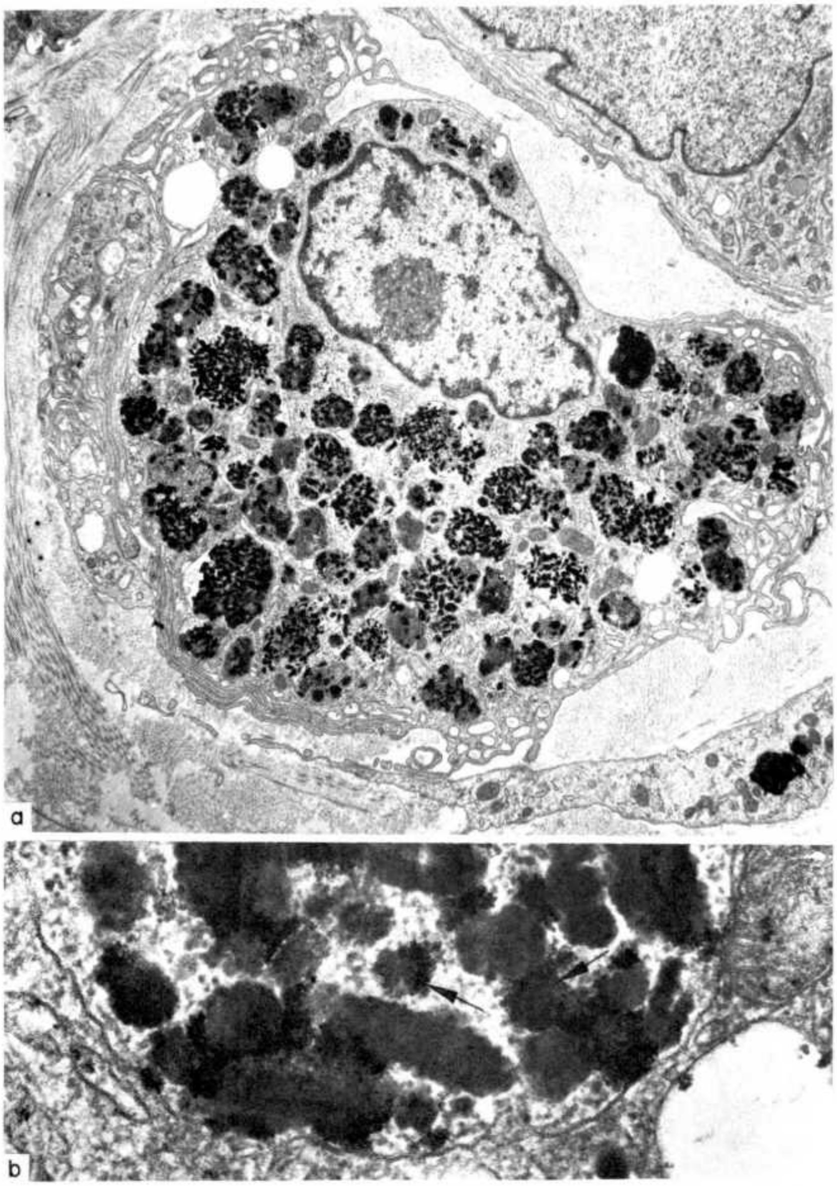

FIGURE 3a. Dermal melanophage packed with phagosomes containing melanosomes, disrupted melanosomes, and smaller electron dense granules (× 7700); 3b. Acid-phosphatase activity (arrows) within phagosomal vacuole of melanophage (× 66,900).

peppered with finely dispersed melanin granules. These cells were DOPA-positive and represented intra-epidermal melanoma cells as revealed by electron microscopy (see below).

Electron microscopy

Identification of cell types. Apart from the melanocytes within the basal layer of the epidermis two types of pigmented cells were identified.

1.) Melanoma cells. These cells were abnormally large and exhibited multilobulated or even multiple nuclei with irregular chromatin patterns; an extensive Golgi system occupied large portions of the cytoplasm which also contained abundant profiles of endoplasmic reticulum and multiple large and polymorphous mitochondria (Zelickson, 1962). Most of these cells contained membrane delimited vacuoles that measured up to 3 μ in diameter and appeared electron microscopically 'empty'. Multiple polymorphous melanosomes of all stages of melanization were dispersed throughout the cytoplasm. Autophagosomes containing aggregated melanosomes were also encountered (Fig. 2a).

Ultrastructural cytochemistry revealed dense reaction product of DOPA-melanin within Golgi cisternae, small Golgi-associated vesicles and stage I and II melanosomes (Fig. 2b). (Some of these cells were found, in serial sections, to be identical with the large tyrosinase-positive cells described in the section on light microscopy.)

2.) Melanophages. These cells were far more numerous and were usually only half the size of the melanoma cells. They showed the structural characteristics of macrophages and were packed with large phagolysosomal vacuoles containing melanosomes, disrupted melanosomes, and smaller electron dense granules and particles (Fig. 3a). In addition, these vacuoles often contained whorls of membranes, lipid droplets, and unidentifiable cytoplasmic debris. The lysosomal nature of these vacuoles was verified by their acid-phosphatase activity (Fig. 3b).

Dermal changes. Blood. Careful screening of the sections revealed the presence of melanoma cells within the lumina of a few capillaries and small precapillary blood vessels (Figs. 4–6). As verified in serial sections, these cells floated free in the vascular channels (Fig. 4) or were partially trapped in niches of the vessels where they appeared to adhere to the surface of endothelial cells (Fig. 5). Others were seen to occlude the capillary. Some of the intravascular melanoma cells were structurally intact and appeared viable (Figs. 4 and 5); they were melanogenically active as shown by the presence of DOPA-reaction product within their pre-melanosomes and Golgi cisternae (Fig. 6). Other intravascular melanoma cells were morphologically damaged and exhibited signs of cytolysis. Lysed melanoma cells were often in intimate contact with the surface of endothelial cells and it is in this respect that an interesting phenomenon was observed; veil-like cytoplasmic projections of endothelial cells were seen folding around and 'embracing' the remnants of lysed melanoma cells that were thus removed from the blood stream (Figs. 7 and 8).

A few macrophages containing ingested melanosomes were also identified within the lumina of dermal blood vessels (Fig. 9) as were isolated melanosomes and cytoplasmic debris (Fig. 4). Both macrophages and isolated melanosomes appeared to float freely among the other blood components or to adhere to the endothelial lining of a capillary. In a few instances a melanin laden macrophage was found surrounded by several concentric basal laminae which suggests that the cell was originally intravascular and that, apparently, the surrounding endothelial cells had degenerated.

Endothelial cells frequently contained phagocytic vacuoles charged with intact or disrupted melanosomes and granular material derived from melanosomes ('melanosome dust') (Figs. 4–7). The melanosomes inside these phagocytic vacuoles were sometimes surrounded by a tightly fitting membrane and were often associated with cellular debris and residues of other organelles. Phagocytosed melanosomes were also observed in pericytes.

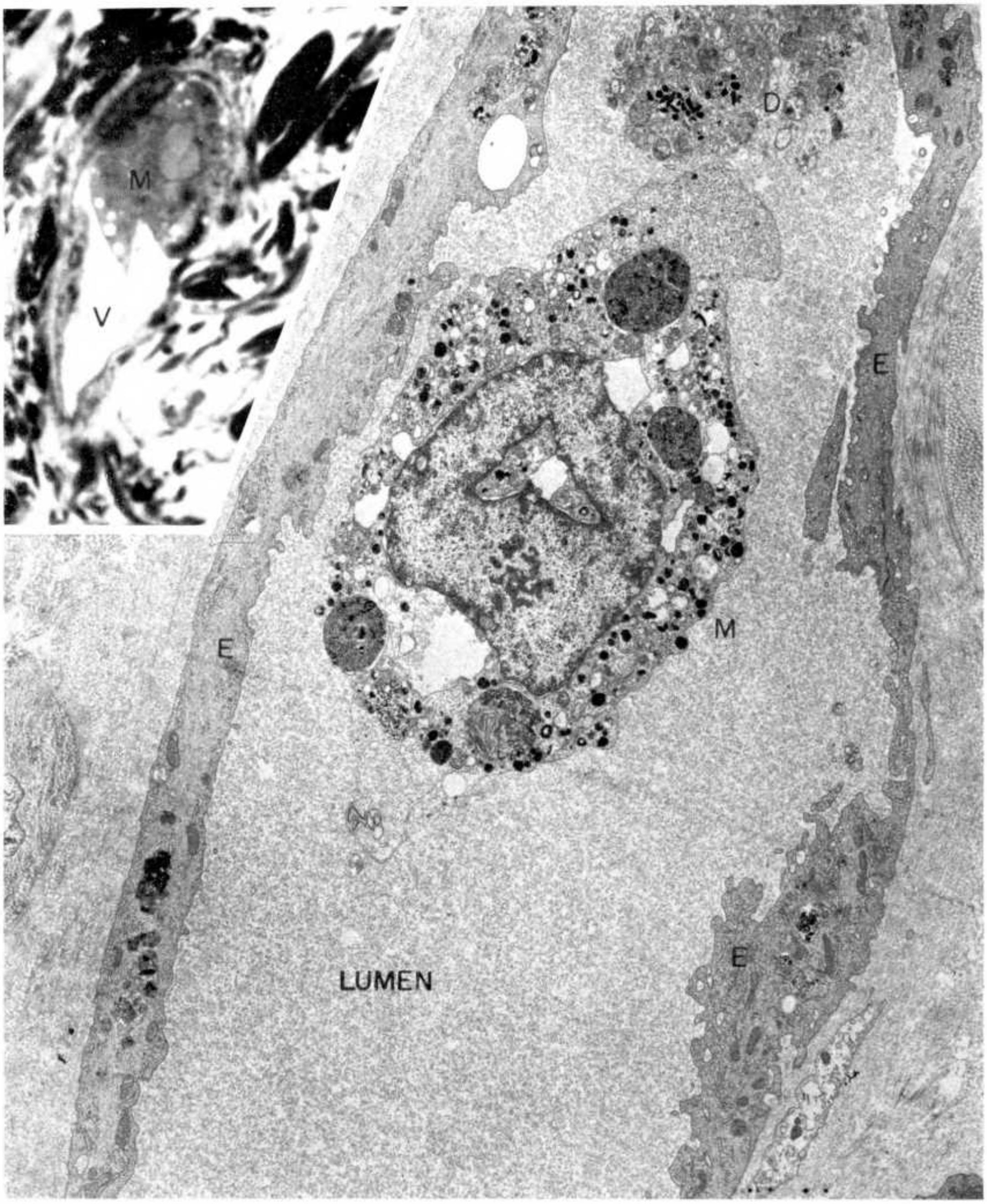

FIGURE 4. Melanoma cell (M) floating free within the lumen of a capillary; E, endothelial cells; D, cytoplasmic debris of another, lysed, melanoma cell (× 4500). Inset: semithin section of Epon-embedded material showing the light microscopic appearance of a similar melanoma cell (M) within a blood vessel (V) (methylene blue, × 1400).

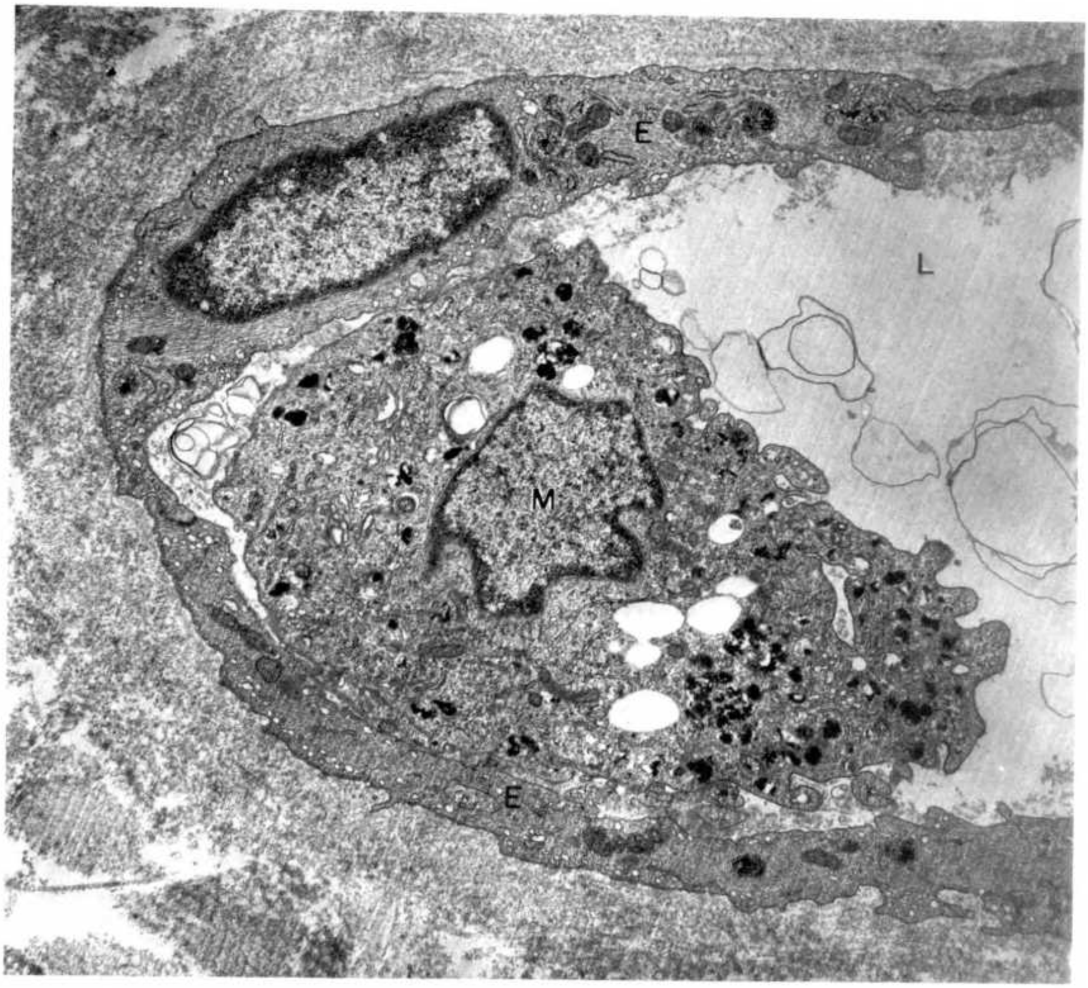

FIGURE 5. Intravascular melanoma cell (M) trapped in a niche of endothelial cells (E); L, lumen (× 11,000).

The extravascular compartment of the dermis was found to have the greatest amount of pigment within the melanophages as was noted with the light microscope (Fig. 3a). These cells were mostly located around blood capillaries or small pre-capillary vessels but were also found in smaller numbers at some distance from vascular channels. The amount of pigment incorporated into individual melanophages varied considerably; some cells contained only a few phagosomes whereas others were literally loaded with melanosome laden vacuoles. Occasional melanoma cells were present among the melanophages both in the vicinity of and distant from blood vessels. They were sometimes surrounded by macrophages or small lymphocytes but were more often found to be dispersed discretely between the fibrous components of the dermis without any apparent association with other cells (Fig. 10).

No amorphous electron dense material was detected in the extracellular compartment of the dermis that could have been interpreted as extracellular oxidation and polymerization product of melanin precursors in the tissue fluid. Extracellular melanosomes were detected only rarely but they were

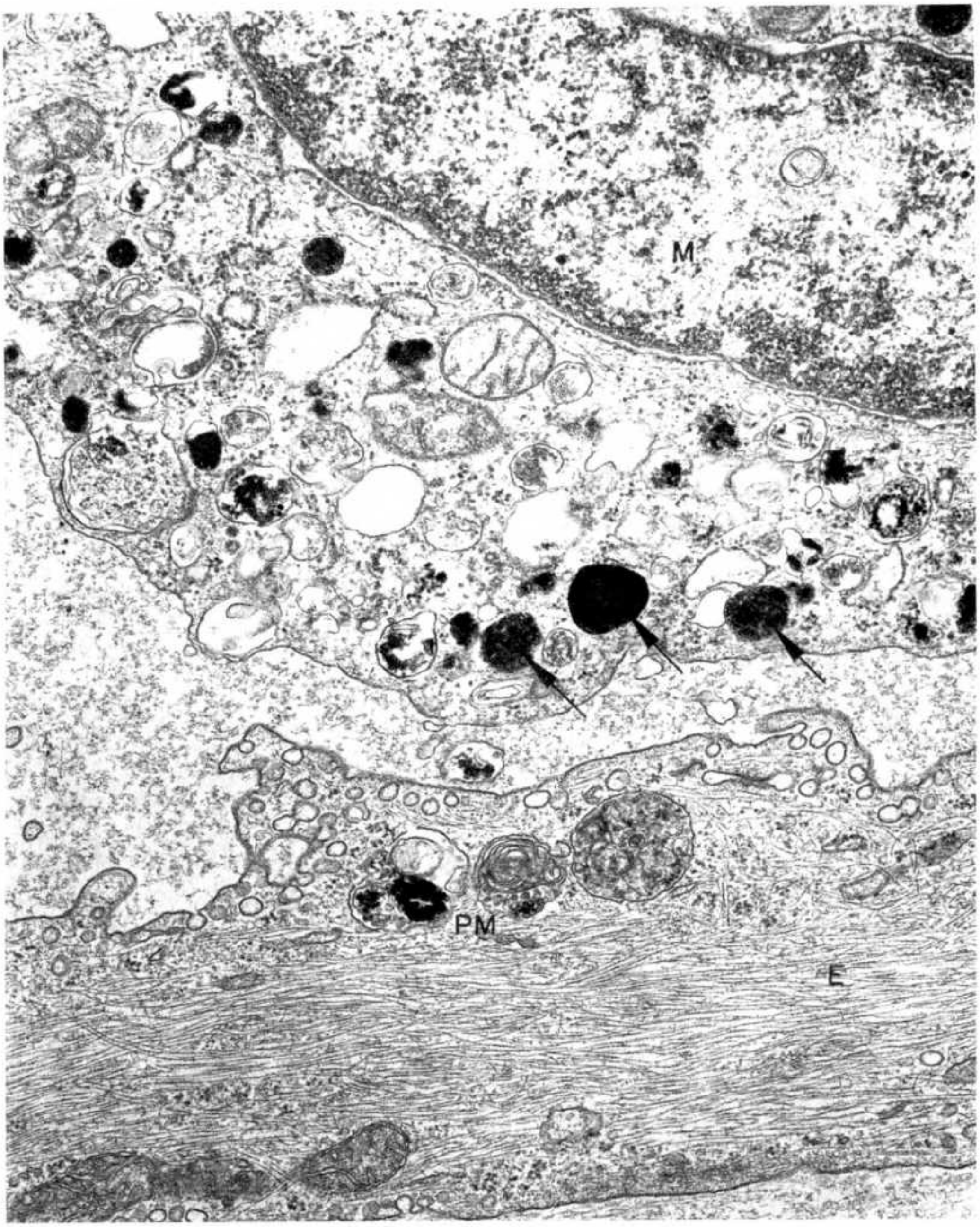

FIGURE 6. Subserial section of Fig. 4. The intravascular melanoma cell (M) exhibits DOPA-activity within melanosomes of various stages (arrows). Phagocytosed melanosomes (PM) are present within phagosomal vacuoles of the endothelial cell (E); L, lumen (× 22,200).

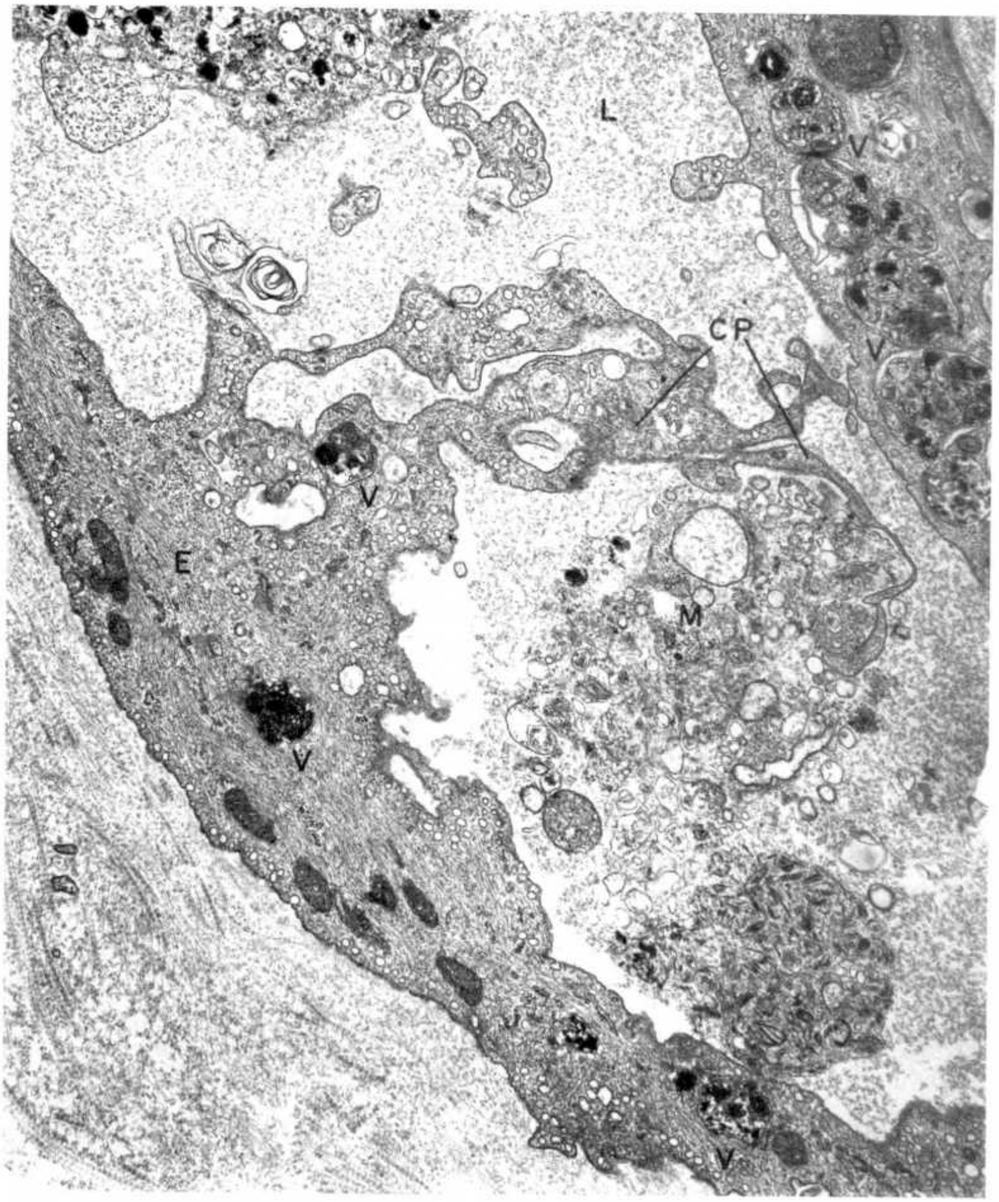

FIGURE 7. Veil-like cytoplasmic projections (CP) of an endothelial cell fold around remnants of a lysed circulating melanoma cell (M). The endothelial cells (E) contain phagocytic vacuoles (V) with intact or disrupted melanosomes; L, lumen (× 11,000).

always very obviously associated with cellular debris, fragments of membranes, and other residues of cell components (Fig. 11a, b). This suggested that they had been released by melanosome-bearing cells, probably melanoma cells, that had lysed. Phagocytosis of such extracellular melanosomes and melanosome debris by melanophages was repeatedly observed (Fig. 11a,b). Phagocytosed melanosomes were also present in Schwann cells and perineural cells.

Epidermis. The general architecture of the epidermis and the morphology of the keratinocytes were normal.

Melanocytes were present in normal numbers. They contained multiple membrane bound round or oval vacuoles measuring 1 to 3 μ in diameter that resembled lipid droplets in shape and size but were delimited by a trilaminar membrane and appeared completely electron lucent (Fig. 12). The melanocytes were otherwise normal and showed all the features of active melanogenesis including large numbers of melanosomes in all stages of development and a well-developed endoplasmic reticulum and Golgi system. Tyrosinase activity was demonstrated in the Golgi cisternae and stage I to III melanosomes.

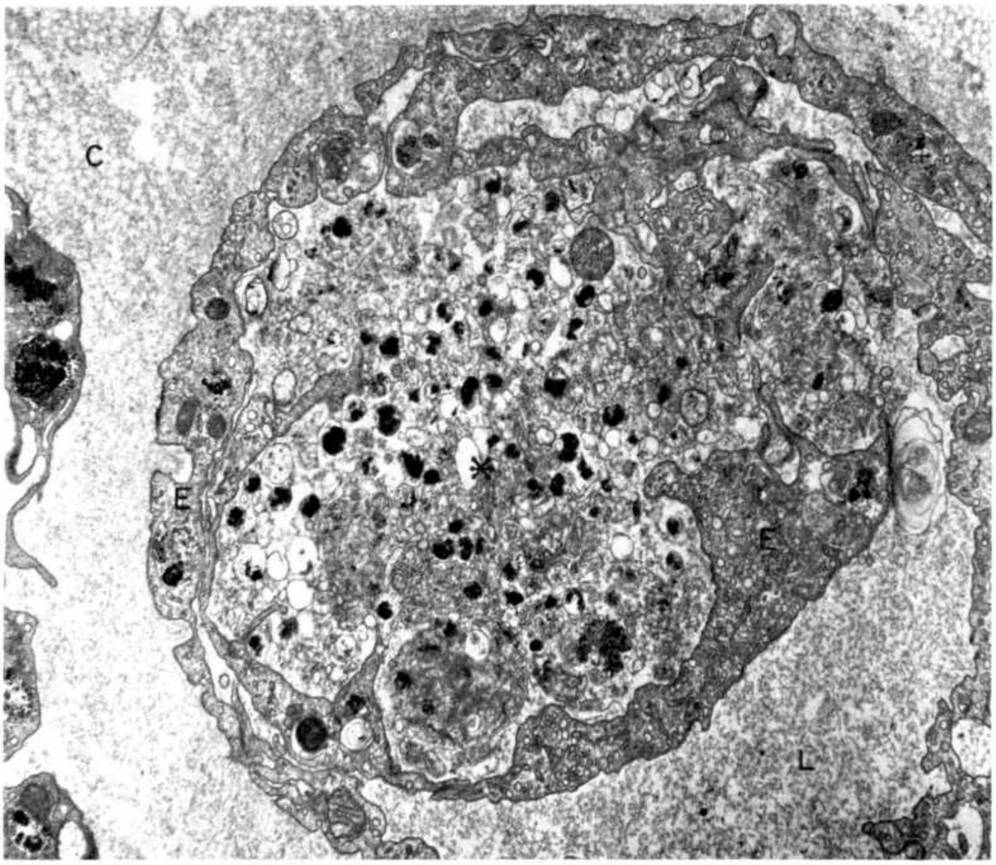

FIGURE 8. The cytoplasmic debris of a lysed melanoma cell (asterisk) is removed from the blood stream by cytoplasmic processes of endothelial cells (E); L, lumen; C, collagen (× 11,000).

There was a definite increase of melanosomes in the keratinocytes of all epidermal layers; many of the basal cells exhibited pronounced supranuclear caps of densely aggregated melanosome complexes.

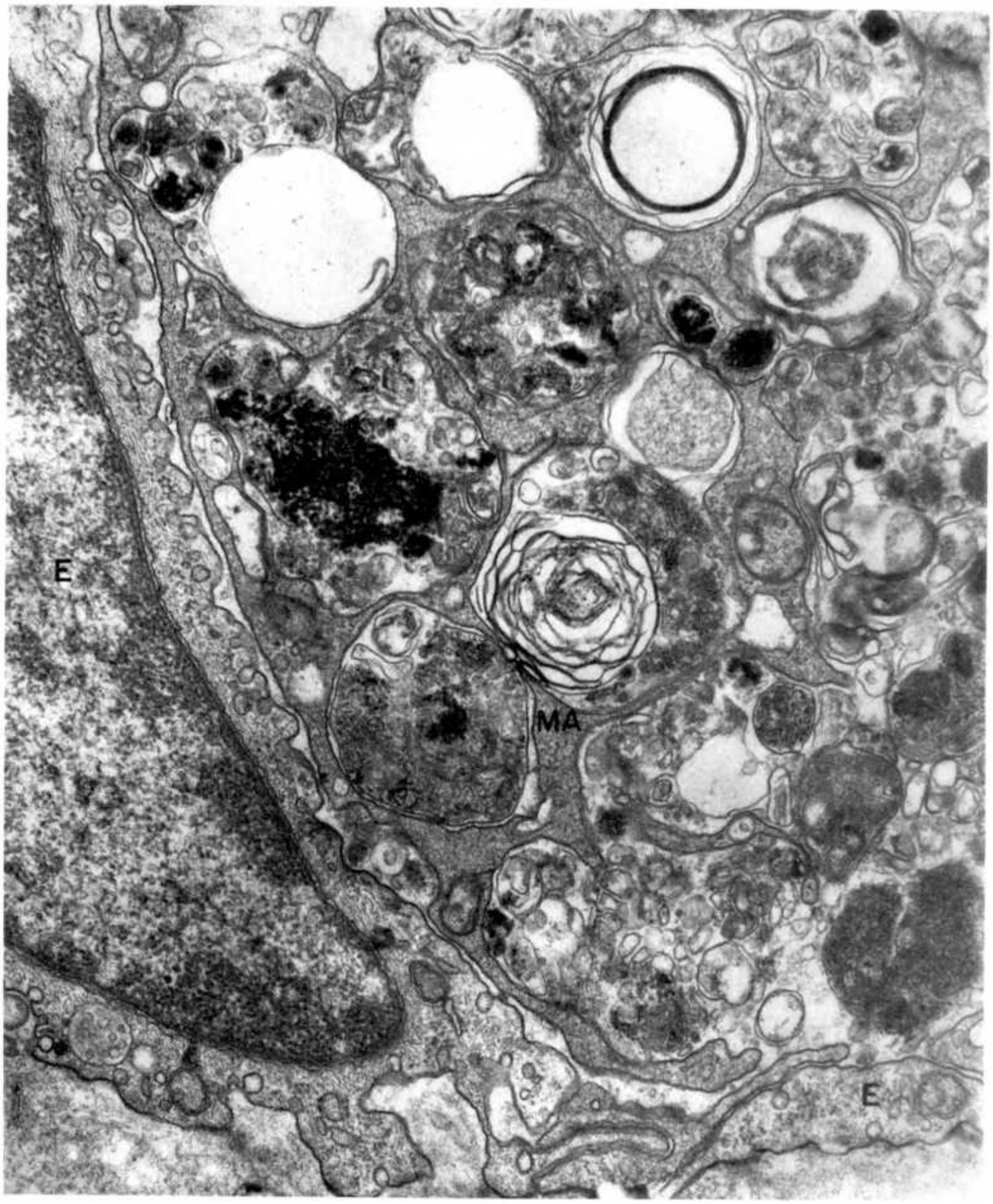

FIGURE 9. Macrophage (MA) within the lumen of dermal blood vessels containing ingested melanosomes and cytoplasmic debris of a lysed melanoma cell; E, endothelial cell (× 22,200).

Lipid droplets were present in some keratinocytes and in some of the otherwise normal Langerhans' cells.

Melanoma cells, as identified by the criteria given above, were occasionally found within the epidermis (Fig. 13). Histiocytes or lymphocytes that had invaded the epidermis were, in rare instances, seen in the vicinity of such tumour cells.

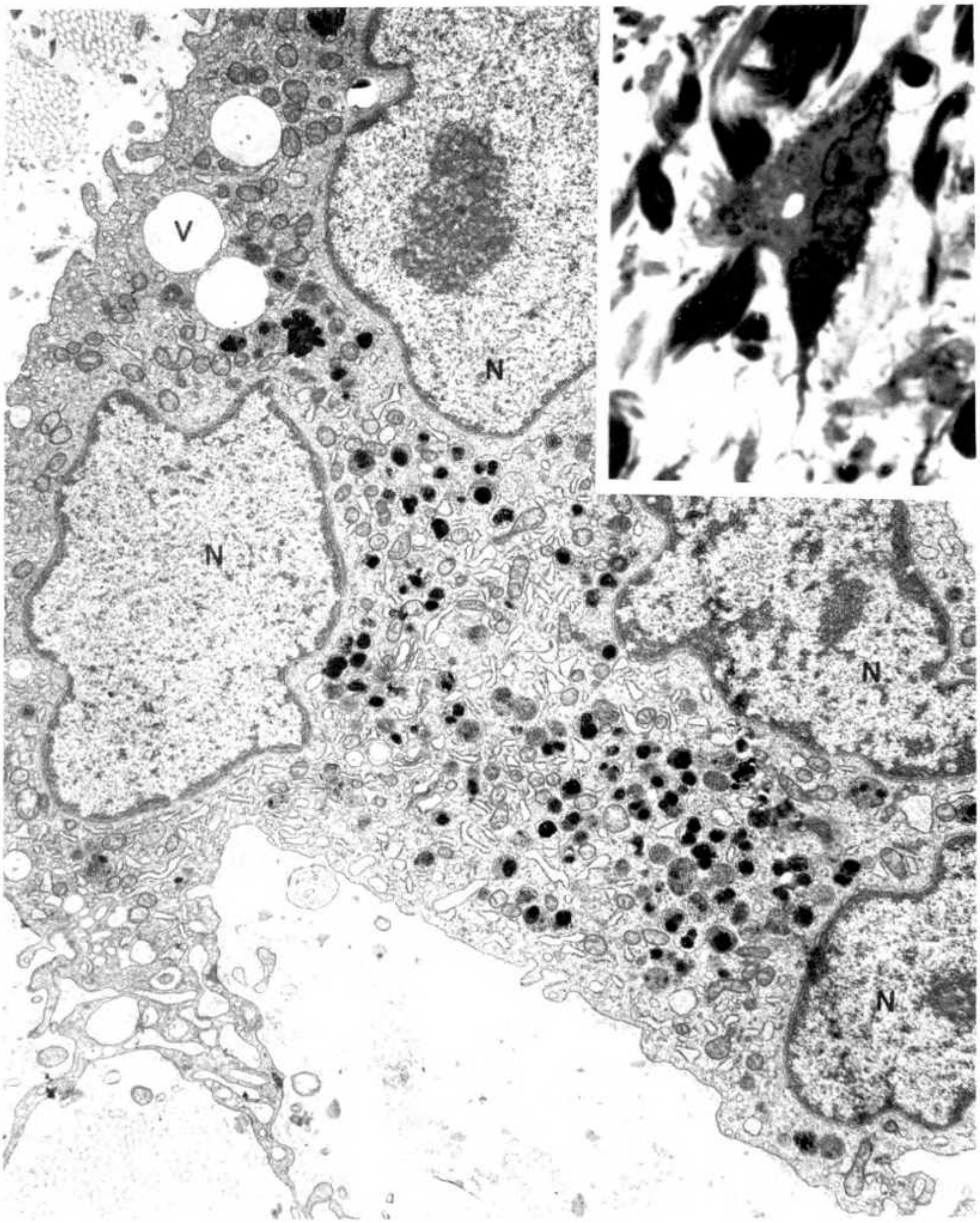

FIGURE 10. One of the melanoma cells dispersed singly within the dermis. N, multiple nuclear profiles; V, clear vacuoles (× 9600). Inset: light microscopic appearance of melanoma cell infiltrating collagen. Semithin section of Epon-embedded tissue (methylene blue, × 1400).

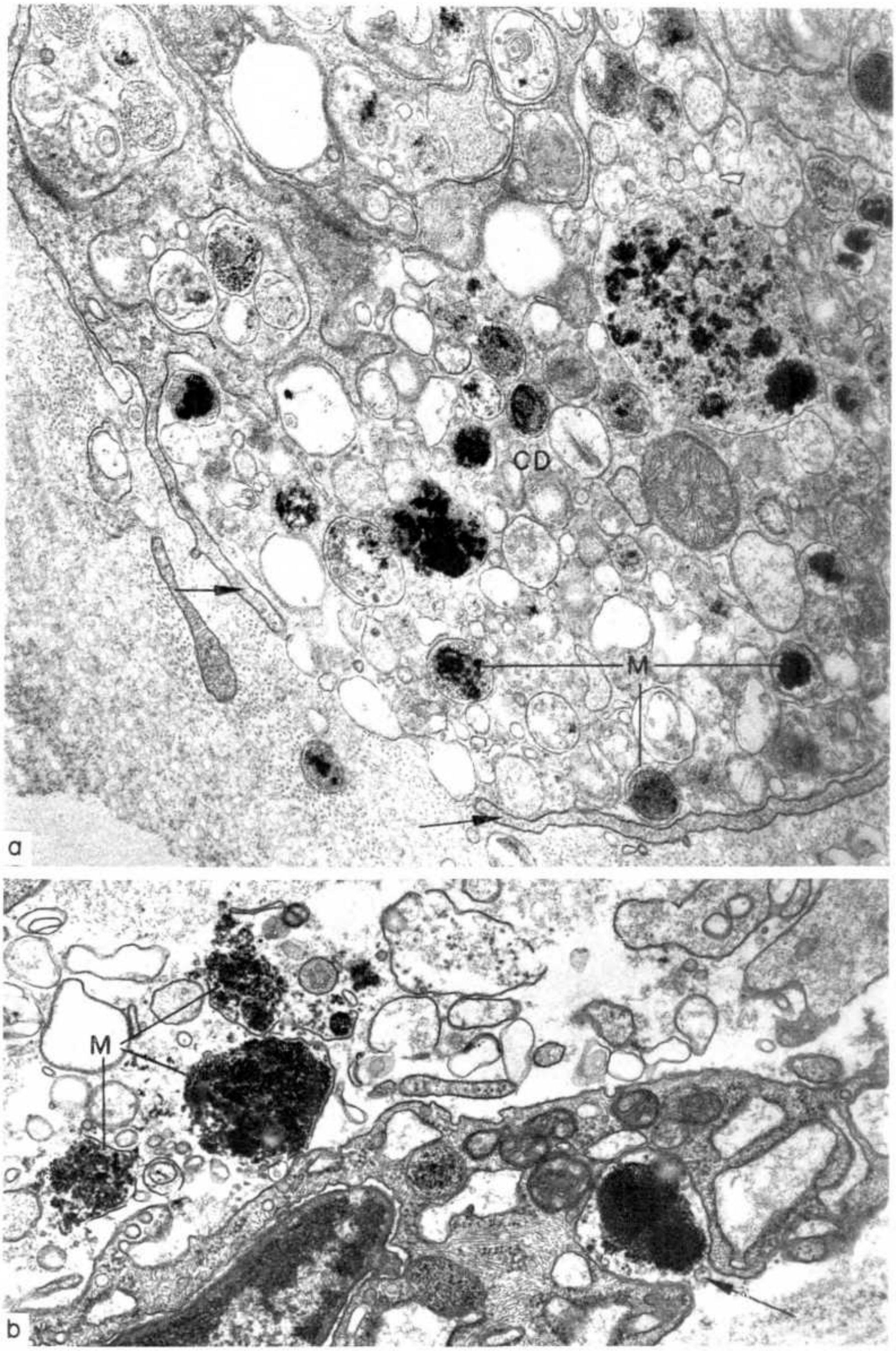

FIGURE 11a. Cytoplasmic projections (arrows) of dermal macrophage phagocytosing melanosomes (M) and cytoplasmic debris (CD) of a lysed melanoma cell (× 22,200); 11b. Uptake (arrow) of extracellular melanosomes probably derived from lytic melanoma cells by a macrophage. Other extracellular melanosomes (M) are clearly associated with cytoplasmic debris (× 22,200).

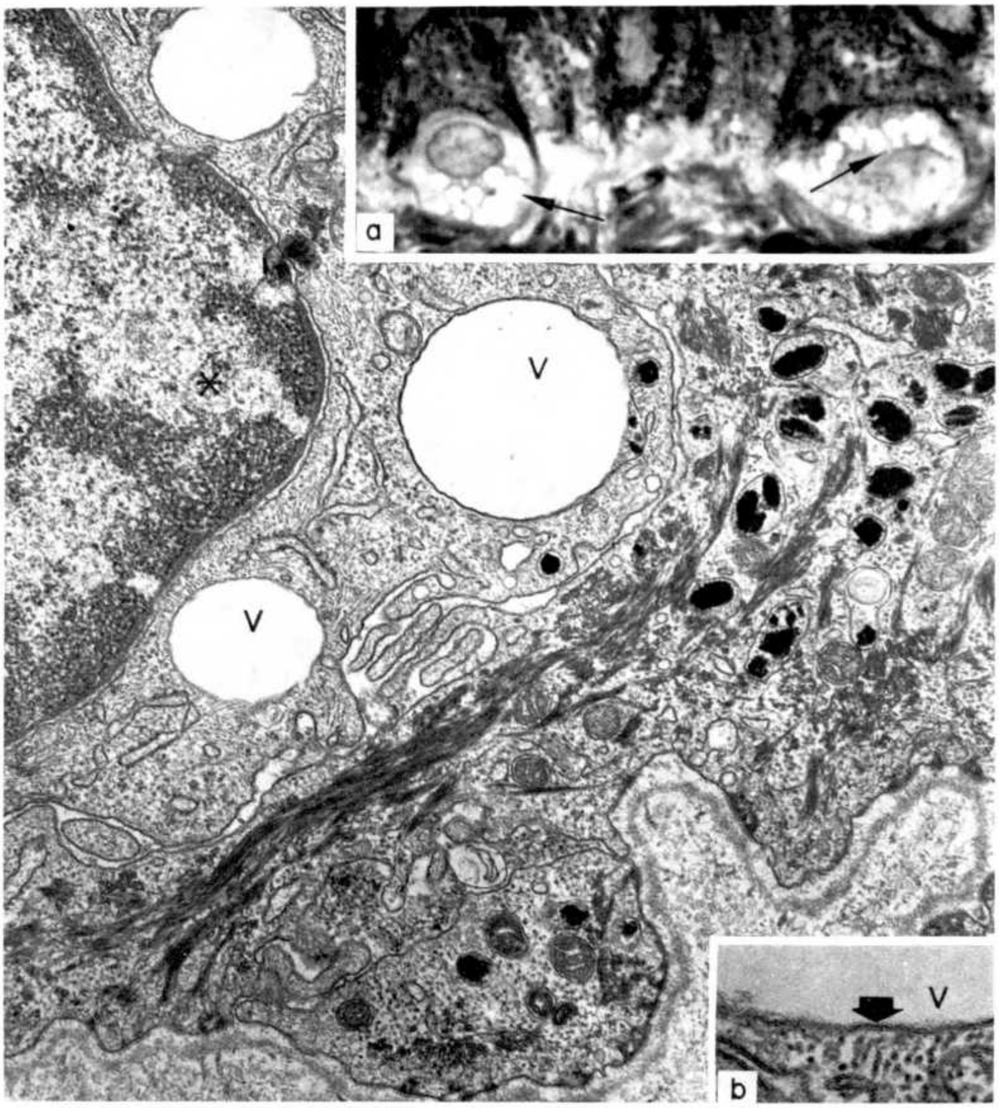

FIGURE 12. Part of an epidermal melanocyte (asterisk). Electron lucent, large membrane-bound cytoplasmic vacuoles (V) are found within the cytoplasm (× 22,200). Inset a: this semithin section is shown to demonstrate epidermal melanocytes with multiple vacuoles (arrows). Methylene blue. (× 1400). Inset b: trilaminar membrane (arrow) surrounding a vacuole (V) (× 82,600).

DISCUSSION

The slate-blue or gunmetal-like pigmentation in patients with diffuse melanosis results from a scattering of light as it passes into the skin and indicates that a major proportion of the responsible pigment is deposited in the dermis (Fitzpatrick *et al.*, 1954). This holds true for a variety of conditions but is most pronounced in argyria (Hönigsmann *et al.*, 1973) and melanoma-associated melanosis (Fitzpatrick *et al.*, 1954). Our results confirm this phenomenon and, in addition, provide some insight

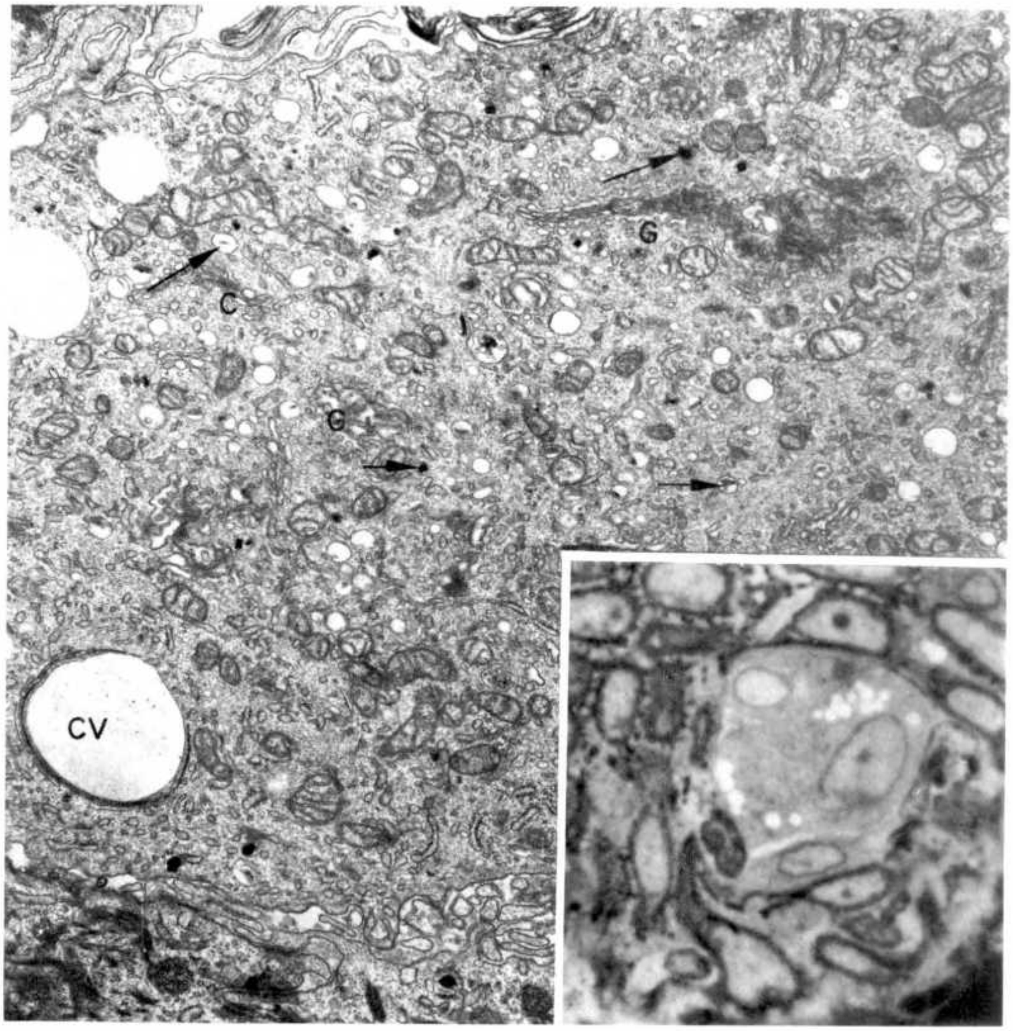

FIGURE 13. The inset shows a melanoma cell within the epidermis as it appears in a semithin section at the light microscope level. The electron micrograph reveals its extensive Golgi apparatus (G) and numerous melanosomes in all stages of maturation (arrows) as well as cytoplasmic vacuoles (CV) (× 11,000).

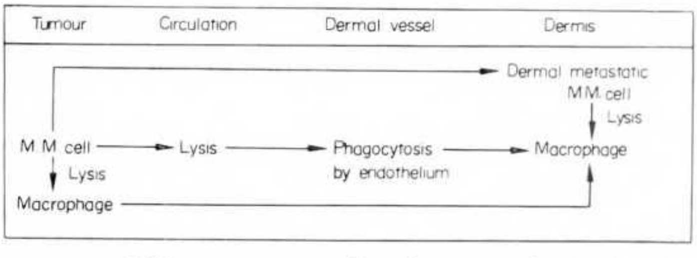

FIGURE 14. Melanosome transport in melanoma-associated melanosis.

into the pathogenesis of the latter condition. The mechanisms involved are complex and encompass several interdependent processes that are all somehow related to the dissemination of tumour cells. The discussion which follows attempts to outline the sequence of events that appear responsible for our patient's melanosis.

Dermis

1.) *Tumour cells* can be demonstrated in the white blood cell (WBC) concentrates of occasional patients with widespread metastatic melanoma and are thus known to circulate in the blood stream (Goodall, Spriggs & Wells, 1961; Sandberg *et al.*, 1958; Alexander & Spriggs, 1960; Pruitt, Hilberg & Kaiser, 1958). In our patient such cells were detected and identified within the terminal blood vessels of the skin both by light and electron microscopy. If it is taken into consideration that histological and ultrathin sections can be representative for only minute volumes of tissue and that intravascular melanoma cells were found repeatedly in sections from different specimens, the total number of circulating melanoma cells must have been large. These intravascular melanoma cells appeared viable and were floating free within the vessels or were trapped in small capillaries; although none was in fact seen to be crossing a capillary wall, their presence in the connective tissue strongly suggests their migration from the vascular lumen into the extravascular compartment. This notion is supported by their distribution, as discrete cells, within the connective tissue and the fact that they were preferentially located around the vessels. Since these cells maintained their melanogenic potentials they represent an important source of continued melanosome production within the dermis. As they degenerated and lysed, their pigment organelles were phagocytosed by and were accumulated within macrophages. Thus a diffuse spread of melanoma cells from the vessels to and throughout the dermis, the continued production of melanosomes within these cells and, finally, the phagocytosis of the released melanosomes by macrophages represent one possible pathway of pigment to the patient's dermis.

2.) *Damaged or lytic melanoma cells* that circulate in the blood stream cannot actively breach and thus cross the wall of a capillary. Such cells were also present within the vessels of our patient, but they were eliminated from the circulation by a mechanism that is illustrated in figs. 7 and 8: a partially lysed melanoma cell is removed to a niche of the capillary lumen by cytoplasmic projections of an endothelial cell which indicates phagocytosis. Isolated melanosomes derived from such cells or liberated from distant tumour cells were similarly removed from the capillary lumen by the phagocytic activity of endothelial cells. The deposition of melanosomes within endothelial cells therefore represents another mechanism by which pigment granules are deposited in the dermis.

The further fate of the melanosomes within the endothelial cells is not quite clear at present but the 'melanosome dust' observed within their phagosomal inclusions indicates that some degradation of melanosomes can take place within the endothelium proper. Possibly, endothelial cells loaded with melanin slough off and are thus carried away into the circulation (von Albertini & Walthard, 1927; Alexander & Spriggs, 1960); or they lyse and become subject to phagocytosis by macrophages. The accumulation of macrophages, literally loaded with melanosomes and cell debris, in the immediate vicinity of the dermal capillaries and of macrophages surrounded by concentric sheets of (originally) endothelial basal lamina suggest that this may have occurred in our patient.

3.) *Circulating blood macrophages* containing ingested melanin have been repeatedly observed in patients with melanoma (Liebmann, 1929; Goodall *et al.*, 1961; Silberberg *et al.*, 1968). Such cells were also present within the vessels of our patient. Macrophages are capable of leaving capillaries by migration and can thus transport phagocytosed melanin into the dermis. It is conceivable that some of the perivascular melanophages were in fact such blood borne cells that had migrated into the perivascular tissue. They represent the third pathway involved in the accumulation of pigment within the skin. A diagrammatic outline of the three mechanisms that appear responsible for the dermal pigmentation in this melanosis is represented in Fig. 14.

Epidermis

Apart from the gunmetal-like colour, the skin of our patient exhibited a definite brown to black hue which was particularly pronounced in the light-exposed areas of the body. This indicates that epidermal hyperpigmentation was also involved in the darkening of this patient's skin and, indeed, the amount of epidermal melanin observed approached that usually seen in the darker races. The ultrastructural features of the melanocytes and their pronounced tyrosinase activity suggests an enhanced melanogenic activity and, since the patient's suprarenals were partially destroyed by melanoma metastases, an increased MSH release by the pituitary may have been responsible for this stimulation (Fitzpatrick & Mihm, 1971; Albeaux-Fernet, Laumonier & Collart, 1949).

The single metastatic melanoma cells that had invaded the epidermis were present only in small numbers so that they could not have contributed significantly to the increase of epidermal pigmentation. Nonetheless, their presence clearly shows that the migration of single tumour cells did not stop at the dermal-epidermal junction and that the epidermis was included in the diffuse single cell metastasization observed in this patient.

The significance of the described vacuoles within the epidermal melanocytes eludes us at present. They are certainly different from and should not be confused with the lipid vacuoles observed in epidermal melanocytes in patients with eruptive xanthoma (Silvers, Becker & Helwig, 1973), Refsum's syndrome (Anton-Lamprecht & Schnyder, 1973; Puissant, Reynes & Duperrat-Noury, 1973) or in melasma (Konrad, 1974 b). They do not reflect a disturbance of lipid metabolism nor do they indicate cell degeneration; further studies appear necessary to clarify the significance of these vacuoles.

Implications

Melanoma-associated melanosis is a rare phenomenon and occurs only in a very small number of patients with advanced metastatic melanoma; to our knowledge only seventeen cases have been reported in the literature (Fitzpatrick *et al.*, 1954; Goodall *et al.*, 1961; Silberberg *et al.*, 1968). As our study is based on only one patient it cannot possibly claim to have outlined a pathogenesis that holds true for all patients and for melanoma-associated melanosis in general. The possibility exists that our patient was unique and, indeed, the only previous ultrastructural study of this condition has failed to reveal a dissemination of melanoma cells within the dermis (Silberberg *et al.*, 1968). However, these cells are so discrete that they may be missed if ultrastructural DOPA-reactions are not performed and this may explain the discrepancy of the results. The possibility therefore exists that there are two types of melanoma-associated melanosis: one resulting from the extracellular oxidation of melanin precursors, as suggested in the elegant study by Fitzpatrick *et al.* (1954); and another one based on the elaboration of pigment organelles by melanoma cells as outlined in the present study.

Nonetheless, all patients reported so far have been strikingly similar. All had diffuse gunmetal-like melanosis of the skin and mucous membranes, multiple metastases and melanuria (Fitzpatrick *et al.*, 1954). It may be significant that there were massive metastases in the heart, including the endocardium, in all patients who were subjected to autopsy (Ritz, 1949) and one could speculate that a continuous dissemination of tumour cells occurred from this organ. It is tempting to speculate further that the diffuse pigmentation of the mucous membranes, the internal organs, and the bone marrow was due to a similar spread of individual tumour cells. Unfortunately, most of the internal organs of our patient were not available for histological studies but the heart, which was examined, was found to be permeated by individually dispersed melanoma cells that had spread in a very similar pattern to that described for the skin. Also, a diffuse infiltration of the liver and spleen by pigmented tumour cells has been reported in another patient (Ritz, 1949).

A third speculation concerns the state of the immunological surveillance and defence systems in our patient. The massive dissemination of individual tumour cells and their apparent 'take' in almost all organs suggest that such defence systems were practically non-existent. The apparent lack of round-cell reactions to the tumour cells supports this conclusion but, unfortunately, during the patient's stay in the hospital no immunological studies were performed that could have provided a more meaningful answer to this question.

ACKNOWLEDGMENTS

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Electron microscopic studies of xeroderma pigmentosum: unusual changes in the keratinocyte

TAKUO TSUJI

Department of Dermatology, Osaka City University Medical School, Abeno, Osaka 545, Japan

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SUMMARY

Two out of sixteen biopsies from verrucous lesions of xeroderma pigmentosum revealed unusual electronmicroscopic changes. Structures resembling anchoring fibrils and the basal lamina were noted within the grossly dilated rough endoplasmic reticulum of some basal and suprabasal keratinocytes.

Xeroderma pigmentosum (XP) is a recessively inherited disorder characterized by the rapid development of ageing and neoplastic changes in exposed skin.

During a study of the ultrastructural changes in various stages of skin lesions of XP, unusual and previously undescribed features were found in epidermal keratinocytes of one verrucous skin lesion. These included an extreme dilatation of the rough endoplasmic reticulum which sometimes contained strange fibrils.

A careful re-examination of other material revealed that the features could be found rarely in similar verrucous skin lesions of XP.

The purpose of this paper is to describe the features and to discuss their significance.

MATERIALS AND METHOD

In the present work, biopsies from five clinically typical cases of XP were examined electron microscopically. They were taken from skin lesions in various stages of development, sixteen of which were verrucous lesions without any malignant features light microscopically.

Specimens were pre-fixed at 4°C for 1 h with 2% glutaraldehyde buffered with 0·2 M phosphate (pH 7·4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0·2 M phosphate (pH 7·4) for 1 h and then dehydrated by graded ethyl alcohol and embedded in Epon 812 according to the method of Luft (1961).

Specimens were cut with a Porter–Blum MT-2 ultramicrotome and stained on the grid for 1 h with a saturated aqueous solution of uranyl acetate followed by immersion for 10 min in Reynold's lead citrate. They were examined with a JEM 7 electron microscope.

RESULTS

In general the ultrastructural changes were similar to those reported by Rasheed *et al.* (1969) and Caputo & Califano (1971). They consisted of shrinkage or enlargement of the keratinocytes and their nuclei, widening of the intercellular spaces, fragmentation of the desmosomes with formation of microvilli-like processes on the surface of the cells, and irregular distribution of the tonofilaments; furthermore, there was a pronounced polymorphism and a definite increase in the number of the melanosomes. The severity and extent of the observed ultrastructural changes depended on the stage of the disease.

The following unusual features were observed in only two of sixteen specimens taken from verrucous lesions.

Clear areas of different sizes and shapes and occupying varying amounts of cytoplasm were observed mainly in basal and lower spinous cells (Fig. 1). Cells containing such areas were present in groups which were separated from one another by numbers of cells containing no clear areas. There was no regular pattern of distribution of cells containing clear areas.

The clear areas were usually filled with a fine granular substance and enclosed by a membranous wall studded with ribosomes on the outer side (Fig. 2). Based on these findings, the clear areas are considered to be dilated rough endoplasmic reticulum.

The dilatation of the rough endoplasmic reticulum appeared to start mainly near the nucleus as oval or irregular vacuoles (Fig. 3). With increasing size they became intricately meshed with the surrounding cytoplasmic elements in multilocular or vermicular shapes (Figs. 1 and 2). In keratinocytes where the dilated rough endoplasmic reticulum occupied most of the cytoplasm, the remnants of cytoplasmic elements were present at the peripheral portions of the cell without any picture of aggregation or compression of the cytoplasmic elements (Figs. 4 and 5). At an early stage of dilatation, a well-developed Golgi complex was often seen close to the dilated rough endoplasmic reticulum but with increasing dilatation, the complex became ill-developed or atrophied. There were no notable changes in the structure of other cytoplasmic organelles when compared with those in cells containing no dilated rough endoplasmic reticulum.

The nuclei were indented or multilobular, and the dilated endoplasmic reticulum frequently conformed in shape to the concave zone formed by depressions in nuclear membranes (Figs. 1, 3 and 4). When the dilated rough endoplasmic reticulum was located near the nucleus, it was sometimes observed to be continuous with the outer nuclear membrane. The nucleoli were usually enlarged and often occupied a large part of the nuclear substance (Fig. 4). However, none of the cells in question was seen in mitosis.

In addition to such characteristic findings, strange fibrils were often observed within the dilated rough endoplasmic reticulum, intermingled with the finely granular substance (Figs. 2 and 5). The fibrils were about 4000 Å in length and had a cross-banding pattern symmetrical about the midpoint (Fig. 6). The cross-striations did not occur in a regularly repeating manner. The fibrils seemed to form a spindle when in a complete form although they were seen in various forms depending on the plane of section. The fibrils were 600 Å in diameter at the central part decreasing to 200 Å at both ends.

The ends of the fibril were attached to an amorphous lamina (about 700 Å thick) composed of a fine granular substance (Figs. 5 and 6).

Adjacent fibrils sometimes interlinked and a characteristic symmetry was seen. Alignment resulted in cross-banding due either to interlinking by common filaments or to association with the same pieces of the amorphous lamina (Figs. 5 and 6).

These strange structures were not seen passing into the intercellular space.

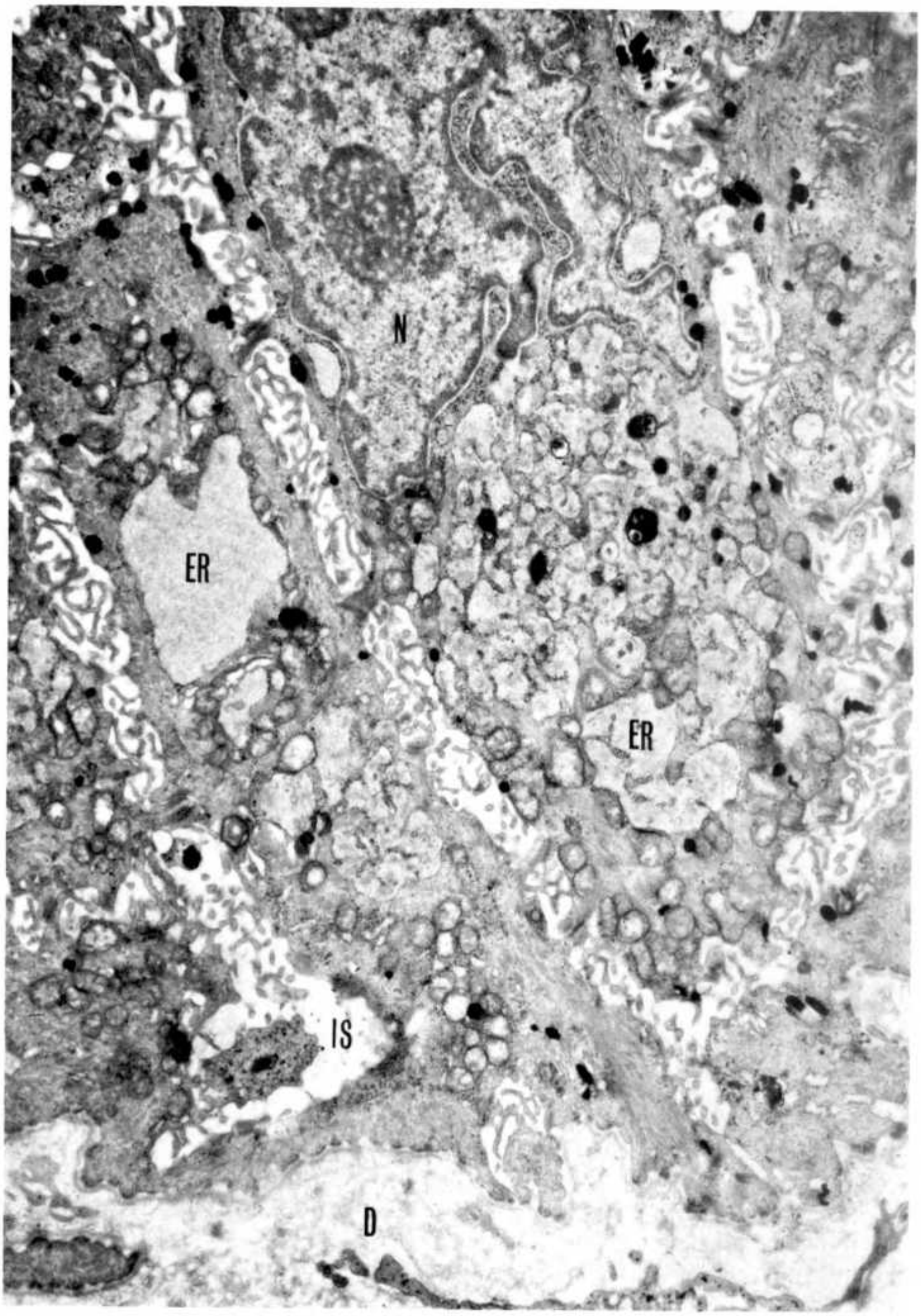

FIGURE 1. Dilated rough endoplasmic reticula (ER) of different sizes and shapes are seen in some of the basal cells. The nucleus (N) of the basal cell is very indented. Widened intercellular spaces (IS) with microvilli are also seen; D, dermis (× 6400).

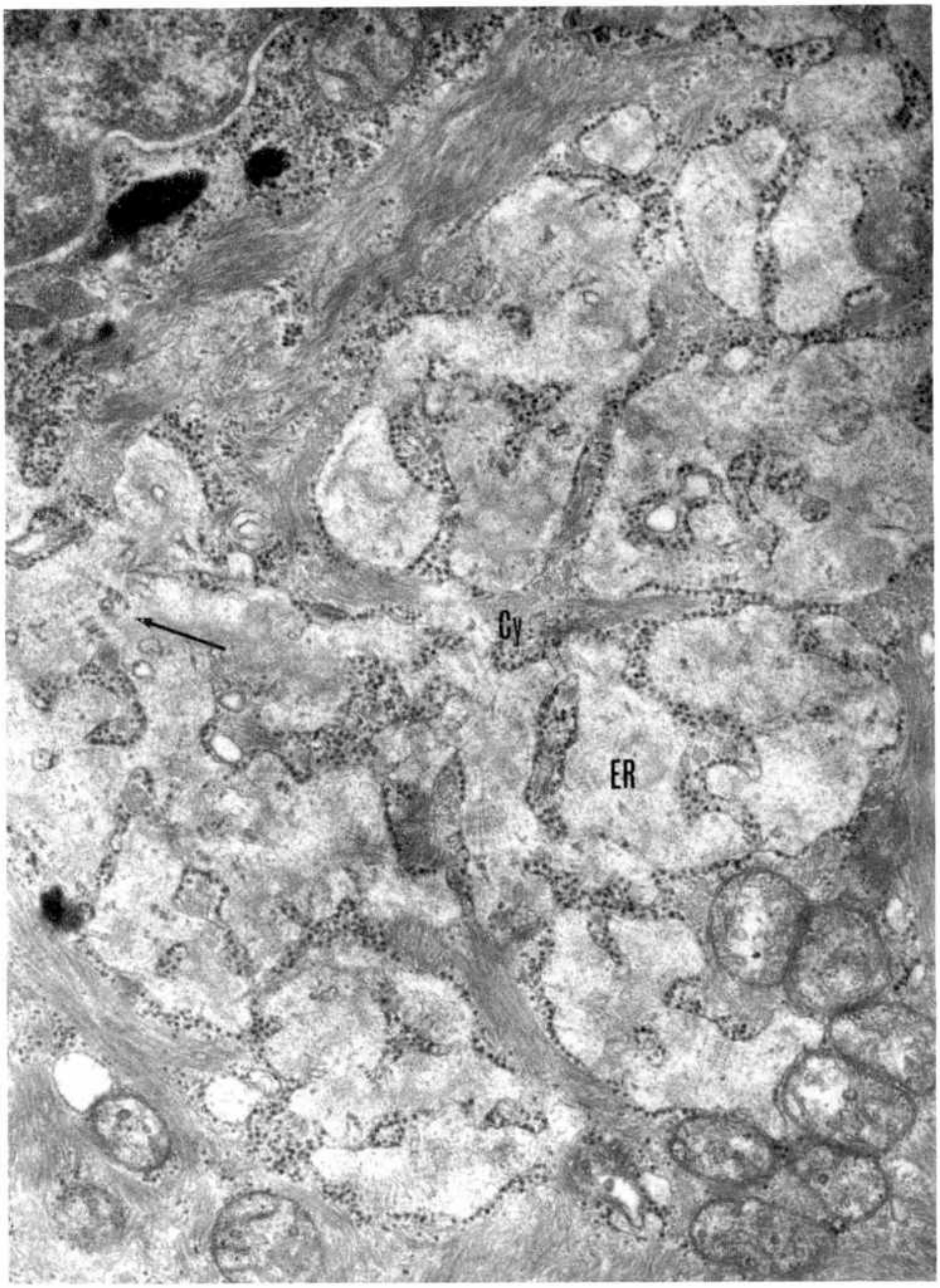

FIGURE 2. Dilated ER communicate with each other. They mesh intricately with the surrounding cytoplasmic elements (Cy) producing a multilocular or vermicular appearance. In some of them, strange fibrils (arrow) are seen within the fine granular substance (× 31,500).

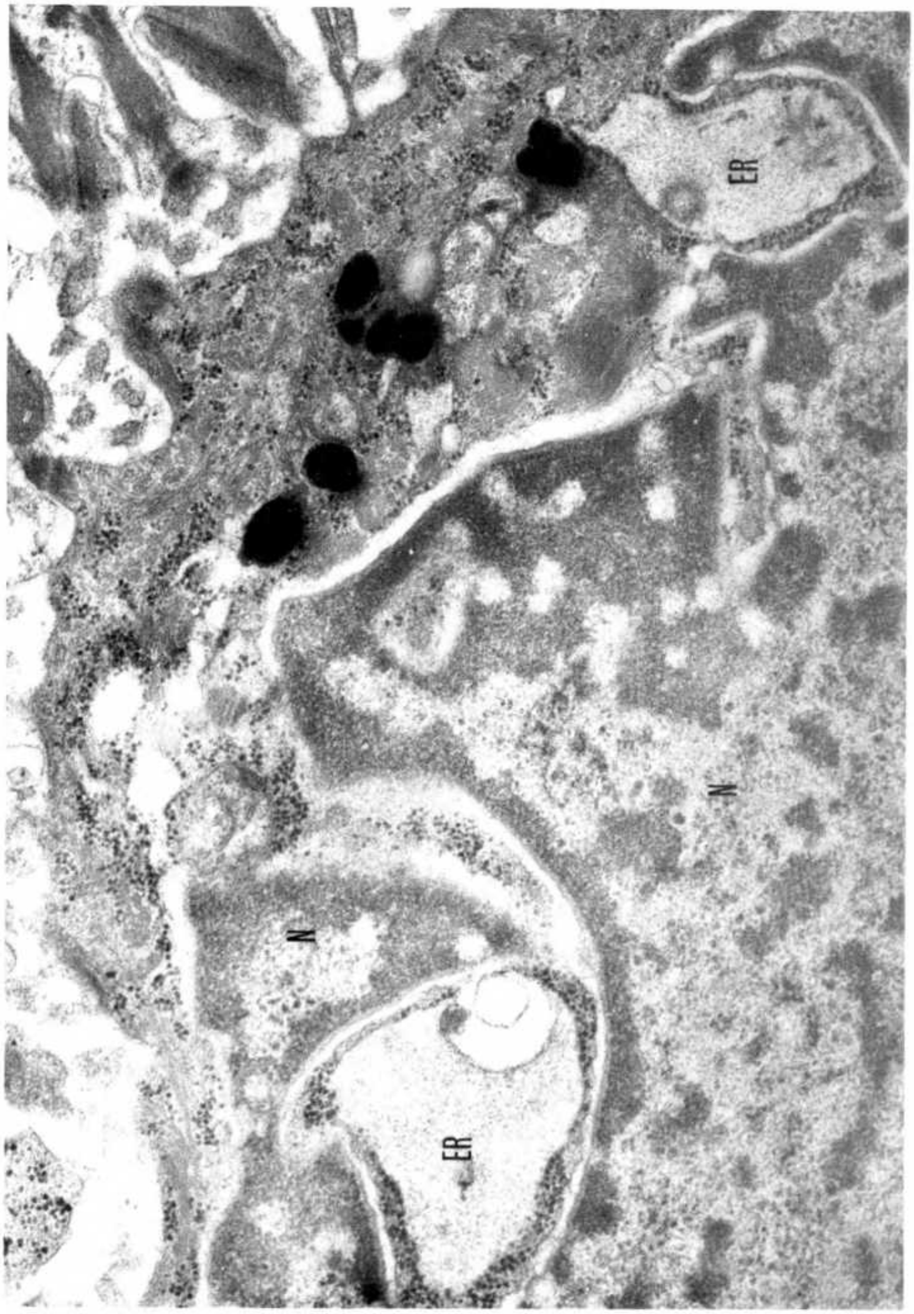

FIGURE 3. Dilated ER are seen close to the nucleus (N). They contain a fine granular substance within them (×35,000).

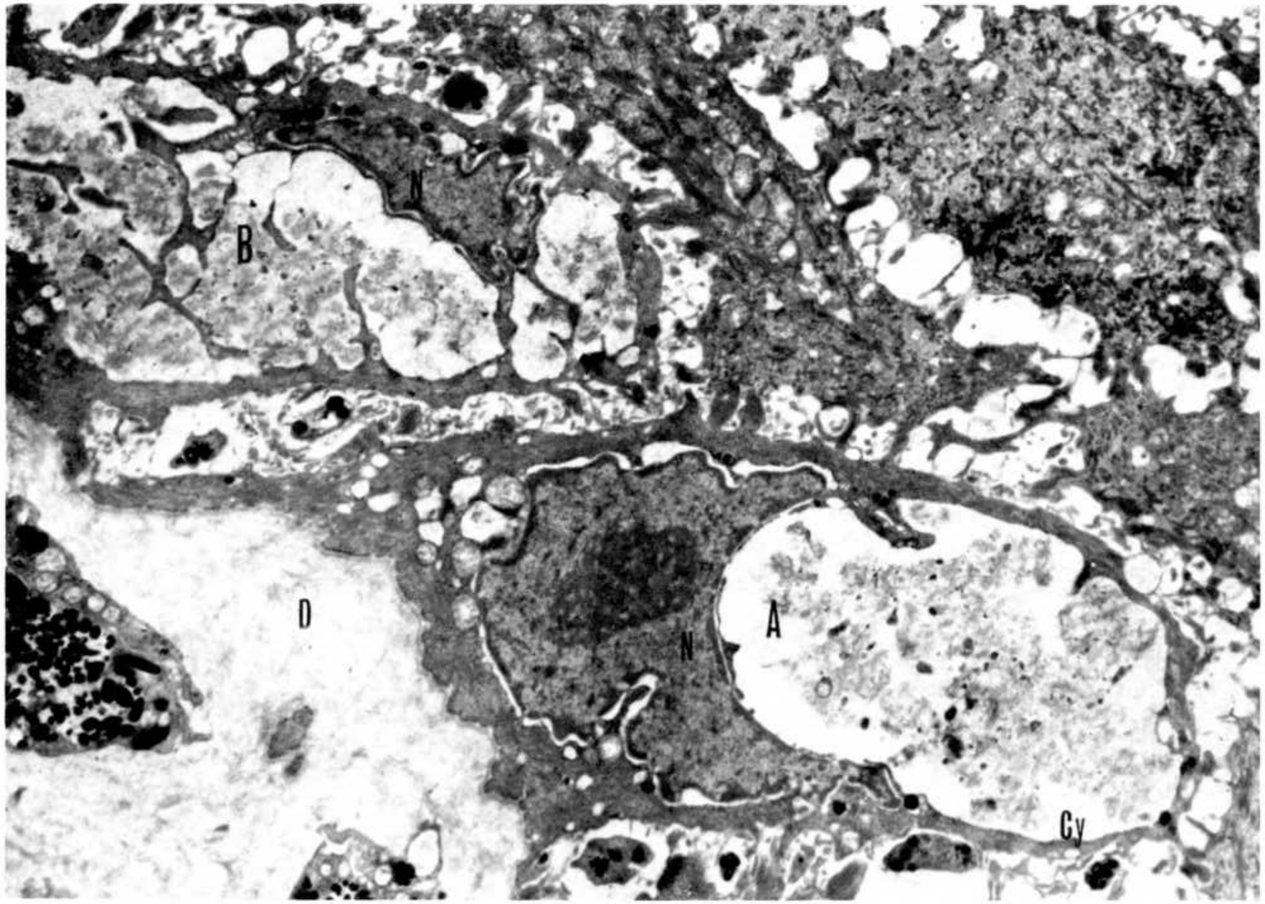

FIGURE 4. Two basal cells showing very severe changes (A and B) are seen. Dilated ER occupy most of the cytoplasmic volume. The remnant of cytoplasmic elements (Cy) is present at the peripheral portions of the cytoplasm. In addition, the nucleus (N) is driven to the corner of a cell, being compressed in appearance (× 7000).

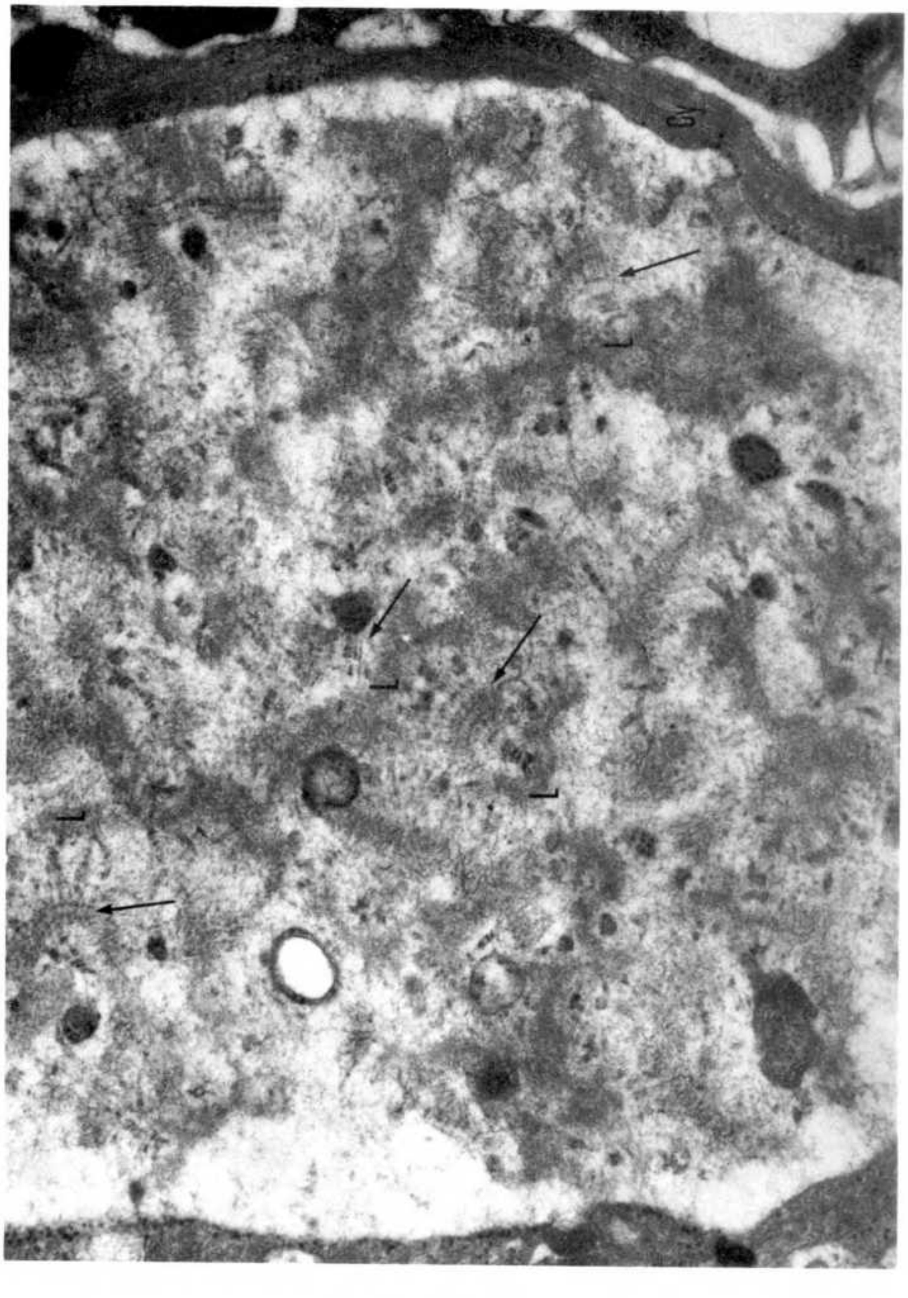

FIGURE 5. Higher magnification of a cell showing severe changes similar to the cells inFig. 4. Strange fibrils (arrows) are scattered within the dilated ER and each end of them is attached to an amorphous lamina (L) (×46,000).

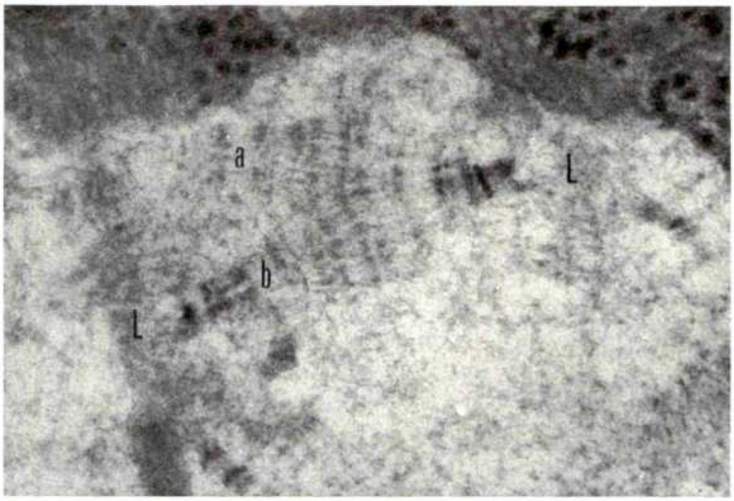

FIGURE 6. High magnification of the fibrils. Two fibrils (a and b) contact each other and their striations align to form longitudinal lines. In addition, each end of these is continous with the common amorphous lamina (L) (× 120,000).

DISCUSSION

The results describe the unusual features seen in the keratinocytes of two verrucous lesions of XP. These are (1) the clear area which is regarded as an extremely dilated rough endoplasmic reticulum, and (2) the strange fibril which is within the clear area and has a cross-banding pattern.

The possibility that the clear area is not dilated rough endoplasmic reticulum, but degenerated cytoplasmic components such as tonofilaments, should be considered. This possibility would be reasonable if one could not find a demarcated wall between the clear area and the other cytoplasmic components. However, it is evident from serial sections of the area that there is a continous membranous wall between them, to the outside of which a layer of ribosomes is adherent.

Another possibility is that the clear areas contain phagocytozed materials. Cells which are actively involved in this process usually contain numerous lysosomes and other vesicles which enclose cell debris. The keratinocytes in question exhibit neither of these ultrastructural features except for melanosome complexes which are observed easily even in the normal keratinocytes. There is little evidence, therefore, that the clear areas result from degeneration of the cytoplasmic components or from phagocytosis of the keratinocyte.

The abnormal dilatation of rough endoplasmic reticulum observed in this work is very unusual as the keratinocyte is a protein-retaining cell (Birbeck & Mercer, 1961) and usually has an ill-developed rough endoplasmic reticulum (Mercer, 1961; Odland & Reed, 1967). It is surmised that such dilatation or rough endoplasmic reticulum containing fibrous structures occured as a part of the process of protein secretion. In other words, the keratinocyte having abnormally dilated rough endoplasmic reticulum may be regarded as a keratinizing cell which has undergone a metaplastic transformation into a protein-secreting cell. Similar metaplastic changes have been reported in epidermal cells of embryonic chicken skin following culture with excess vitamin A (Fell, 1962; Jackson & Fell, 1963).

A few reports demonstrated cytoplasmic changes similar to the clear areas. These include large vacuoles reported by Nix *et al.* (1965) in normal skin irradiated with ultra-violet light, small dilated rough endoplasmic reticulum by Caputo & Califano (1971) in the keratinocytes of desquamating and dyskeratotic lesions of XP, and a hydropic dilatation of rough endoplasmic reticulum by Klehr & Klingmüller (1972). These vacuoles or reticula contain varying amounts of a fine granular substance, but such fibrous structures as seen in the present work have not been found within them.

The strange fibrils seen in the dilated rough endoplasmic reticulum are similar in several structural aspects to the anchoring fibrils (Palade & Farquhar, 1965; Rowlett, 1969) seen immediately under the basal lamina of the epidermis and various other epithelia. The two substances resemble each other in length, width, characteristics of cross-striation and interlinking by common filaments. In addition, each end of these is attached to a basal or amorphous lamina. These structural resemblances of the two suggest that the strange fibrils may be identical to anchoring fibrils, and also suggest that the amorphous lamina may be identical to the basal lamina.

There is only one report dealing with the formation and origin of anchoring fibrils, and that supports the concept of their dermal origin (Briggaman, Dalldorf & Wheeler, 1971). On the other hand, many reports suggest an epithelial origin of the basal lamina (Pierce *et al.*, 1962; Hay & Revel, 1963; Pierce *et al.*, 1964; Hay, 1964; Odland & Ross, 1968; Croft & Tarin, 1970; Briggaman *et al.*, 1971). The present findings suggest that both anchoring fibrils and the basal lamina may be epidermal origin.

In fact, there is some evidence that epithelia may produce collagen and excrete it into the subepithelial space. Trelstand (1971) reported that vacuoles in embryonic chick corneal epithelium contained collagen which was being concentrated and excreted by the cell. Similarly, Carlson (1973) has shown a collagen-like, periodic fibrillar material within membrane-bound bodies in the chick notochordal epithelium. Furthermore, several investigators (Hay, Fleischmajer & Billingham, 1968; Low, 1968; O'Connell & Low, 1970) have suggested that boundary basement membrane and other extracellular connective tissue material of the notochordal and corneal epithelium are of epithelial origin.

The factor which brings about these unusual changes in XP epidermis is unclear. Judging from the fact that the basic disability of XP is a marked sensitivity to sunlight, a possibility is that they are due to solar irradiation. Another possibility is that these changes have some relationship with carcinogenesis, since verrucous lesions of XP in which these unusual changes are found are considered to be a precancerous state.

Recently, it has been shown that there is defective DNA repair in cells of XP patients damaged by solar irradiation (Reed *et al.*, 1969; Cleaver, 1970; Epstein *et al.*, 1970; Burk *et al.*, 1971; Robbins & Kraemer, 1972). Various metabolic disturbances including those involving amino acid metabolism have been noted in patients with this disease (El-Hefnawi, El-Nabawi & El-Hawary, 1962a, b; El-Hefnawi *et al.*, 1963; Moss, 1965). It is possible that such factors may have something to do with the abnormal findings observed in the present work.

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The morphology and longevity of cells derived from primary cultures of guinea-pig dorsal skin cells

C.PROTTEY, P.W.A.TOVELL AND T.F.M.FERGUSON

Unilever Research, Colworth Welwyn Laboratory, Colworth House, Sharnbrook,
Bedford MK44 1LQ

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SUMMARY

Primary cultures of cells harvested from guinea-pig dorsal epidermis were maintained *in vitro* for extended periods. The cells which initially attached to the culture vessels within the first 24 h were epidermal basal cells, but it was generally observed under the culture conditions utilized that these cells possessed limited viability, and would subsequently detach and die within the first week of culture. During this time the cultures became overgrown with fusiform cells, closely resembling fibroblasts, which subsequently required regular subculturing. Electron microscopic examination of the fusiform cells revealed that they did not resemble freshly isolated epidermal basal cells; comparison with true fibroblasts derived from guinea-pig bone marrow strongly suggested that the rapidly proliferating cells were indeed fibroblasts. Demonstration of their ability to synthesize collagen *in vitro* supported this suggestion. These findings are contrary to those widely reported by others for cell cultures derived from guinea-pig auricular epidermis, in which the basal cells have extended viability *in vitro*, and overgrowth by fibroblasts is seldom seen.

Studies on the identity and characteristics of the rapidly proliferating cell cultures derived from guinea-pig ear keratinocytes are well documented. Cruickshank, Cooper & Hooper (1960) were the first to describe how those cells seen in primary cultures of epidermal cells, namely basal cells and melanocytes, possessed limited survival times *in vitro* in their original morphology. With successive subcultures they observed flattened fusiform cells which rapidly multiplied and were morphologically quite unlike those of the initial preparations. These authors concluded that they were dedifferentiated epithelial cells. This work has subsequently been supported by others (Wikswo & Szabo, 1971; Pruniéras, Delescluse & Regnier, 1969; Fritsch & Diem, 1972). Jacquemont & Pruniéras (1969) suggested, on the basis of a specific stain for connective tissue cells (naphthylamidase), that the fusiform cells which are observed to dominate later cultures, and which morphologically resemble true mesenchymal fibroblasts *in vitro*, are indeed keratinocytes which have 'dedifferentiated' to fusiform cells under conditions of culture. Also, Constable (1972) has described epidermal cells in culture from guinea-pig ear which exhibit selective modification of some morphological features but with the retention of characteristically epithelial structures (desmosomes) for up to 12 days in culture. Recently, Regnier, Delescluse & Pruniéras (1973) have reported that two distinct types of cells may be derived from guinea-pig ear skin, namely, true keratinocytes which partially retain their specific fibre–desmosome apparatus but which are slow to replicate *in vitro*, and true fibroblasts from the dermis of the ear. The latter closely resemble bone-marrow fibroblasts in culture, and actively replicate *in vitro*.

Unlike guinea-pig ear, papers describing the behaviour in culture of epidermal cells from the dorsal skin of this species are lacking. As we are currently using guinea-pig dorsal skin as a source of far greater quantities of basal cells than may be harvested from ear skin for studies on epidermal cell metabolism, we have first studied the morphology and behaviour in culture of dorsal skin keratinocytes. These findings are reported and discussed in relation to existing data for guinea-pig auricular epidermal cells *in vitro*.

MATERIALS AND METHODS

Materials

Specialized tissue culture constituents and their sources of purchase were as follows: benzyl penicillin BP and dihydrostreptomycin sulphate BP (Dista Products Ltd, Liverpool); nystatin (E.R.Squibb & Sons, Liverpool); trypsin, ex hog pancreas (Hopkin and Williams, Essex); calf and fetal calf serum (Flow Laboratories, Irvine, Scotland); Hank's balanced salt solution and Eagle's tissue culture medium (Wellcome Reagents Ltd, Beckenham); heparin (Boots, Nottingham).

Radioactive compounds were obtained from the Radiochemical Centre, Amersham, Bucks; the sterile aqueous solutions were added directly to cultures.

Guinea-pigs were approximately 300 g in weight and of the Dunkin–Hartley strain, maintained on R11 diet (BOCM, Silcock), hay and cabbage *ad lib.* Male animals were used throughout the study.

The Castroviejo Electrokeratotome was purchased from Stortz Instruments Ltd, St Louis, Missouri.

Preparation of isolated epidermal cells

Guinea-pigs were killed and their backs were closely shaved over an extended area. The shaved dorsal skin and the surrounding areas of hair were liberally swabbed with 70% alcohol. The excised skins were placed, epidermis down, on a sterile gauze soaked in 'PBS minus' containing a suspension of nystatin (100 mg/l) for 30 min. 'PBS minus' is Dulbecco's phosphate buffered saline (Dulbecco & Vogt, 1954) minus calcium and magnesium ions (Willmer, 1965), but containing penicillin, streptomycin and heparin at strengths of 200 iu/ml, 100 iu/ml, and 10 iu/ml respectively. After the anti-fungal pre-treatment, the skins were transferred to sterile dry petri dishes prior to cutting 200 μm keratotome sections, with a Castroviejo Electrokeratotome, fitted with a 200 μm shim (Blank, Rosenberg & Sarkany, 1961). The Keratotome slices were then floated, epidermis down, on 'PBS minus' containing nystatin (100 mg/ml) for a further 30 min as an added anti-fungal treatment. After 30 min the slices were placed epidermis up, on a sterile, dry petri dish and were floated on a 0·25% solution of trypsin in 'PBS minus' at 37°C for 35 min in order to loosen the epidermis.

After 35 min, the trypsin solution was replaced with 'PBS minus' before separating the epidermis from the dermis. The combined sheets of epidermis were further digested in a 0·25% solution of trypsin in 'PBS minus' at 37°C for 30 min with continual stirring in order to detach the basal cells. The action of the trypsin was stopped by the addition of 1/5 volume of fetal bovine serum. The resultant cell suspension was withdrawn from the remaining epidermis and was filtered through a sterile gauze and centrifuged at 1250 rev/min (approx. 200 *g*) for 10 min at 4°C. The supernatant was discarded and the cells resuspended in 2 ml fetal bovine serum, centrifuged once more, and the supernatant discarded. The cells were recovered as a creamy-white pellet.

Culture of epidermal cells in vitro

The cell pellets prepared as described above were resuspended in Eagle's medium (Eagle, 1959), containing penicillin (20 iu/ml) and streptomycin (100 iu/ml), 10% v/v fetal bovine serum and 10% v/v guinea-pig serum. Aliquots were taken for the determination of cell numbers using a Neubauer Counting Chamber. The volume of cell suspensions was adjusted to give cell densities of approximately 2×10^6 cells per ml. Further aliquots were taken for histological examination by cell smear and for assessment of viability by the dye exclusion technique using Trypan Blue (Wiepjes & Prob, 1970). An equal volume of a 0·25% solution of Trypan Blue in PBS minus was added to 0·5 ml cell suspension and the two were thoroughly mixed and allowed to stand for 10 min. The relative proportions of stained (dead) and unstained (viable) cells were counted in the counting chamber.

Portions, each being 1 ml of the cell suspension, were added to Leighton tubes containing glass coverslips, gassed initially with 5% CO_2 in air, stoppered with sterile bungs, and maintained at 37°C. The cell cultures were fed every 2 or 3 days by replacing with fresh medium and regassing with 5% CO_2 in air.

When the cells had spread over the coverslip in an almost continuous sheet, which generally took about 3 weeks, the cultures were subdivided as follows. The cells were first washed with 'PBS minus' and then incubated with 1 ml 0·5% trypsin in 'PBS minus' at 37°C for 5–10 min, until all the cells had become detached from the coverslips. Any clumps of cells were separated by gently pumping the cell suspension up and down in a pasteur pipette. The cell suspension was transferred to a centrifuge tube and 1 ml fetal bovine serum added to inhibit further trypsinization. The cells were centrifuged at 200 *g* for 10 min and the supernatant discarded. Finally, the cells were resuspended in 2 ml fresh medium, added to two Leighton tubes, regassed and maintained at 37°C. Each Leighton tube now contained one half of the first culture representing a one-in-two dilution.

Preparation of bone marrow fibroblasts

Guinea-pig bone marrow fibroblasts were prepared using the method described by Friedenstein, Chailakhjam & Lalykona (1970). One adult male guinea-pig, weighing 600 g, was killed and the ventral and pelvic areas were shaved and swabbed with 70% alcohol. The rear legs were removed at the pelvis and the femurs were carefully dissected out and opened at each end. Each femur was flushed out with 10 ml of 'PBS minus'. The combined bone marrow cell suspensions were filtered through three layers of gauze and centrifuged at 200 *g* and the supernatant discarded.

The cells were resuspended in Parker's 199 medium containing 20% fetal bovine serum. Six Leighton tubes were each inoculated with 1·5 ml cell suspension, gassed with 5% CO_2 in air and incubated at 37°C. At this dilution the cell density was approximately 10^7 cells per 1·5 ml, but cell counts were of little value as the cell population was predominantly blood cells and macrophages. Fibroblasts were not recognized until after 9–12 days in culture. For this same reason cell viabilities were not assayed.

Incorporation of [U-^{14}C] L-*proline into cells in culture*

Cultures of cells derived from both skin and bone marrow were incubated with 10 μCi [U-^{14}C] L-proline for 24 h in order to assay collagen synthesis. The skin cells were 47 days old and had been through two subcultures whereas the fibroblasts of bone marrow origin were 34 days old and had been through one subculture. Both were confluent cultures in 4 oz. medical flat bottles covered by 10 ml Eagle's medium containing 10% fetal bovine serum and 10% guinea-pig serum, to which 10 μCi [U-^{14}C] L-proline was added. Specific cofactors for collagen synthesis were added to the media, namely sodium ascorbate (1·0 mM), α-ketoglutarate (1·0 mM) and ferrous ammonium sulphate (0·1 mM); the concentrations were as used by Miller & Udenfriend (1970). The reactions were terminated after 2 h by freezing in an acetone/solid CO_2 mixture.

Before extracting the collagen, the protein was first denatured by the addition of ice-cold 10%

trichloracetic acid containing 0·5% sodium tungstate (Miller & Udenfriend, 1970). The residue was then heated with 5% trichloracetic acid in a boiling water bath to extract the collagen. The extract was hydrolysed by heating overnight with 6 N HCl in sealed tubes at 130°C. The resultant amino acids were examined by two-dimensional chromatography on Whatman No. 1 paper using phenol:ammonia:water (160:1:10, w/v/v) for the solvent in the first direction and butanol:acetic acid:water (120:30:50, v/v/v) for the solvent in the second direction. Proline and hydroxyproline were detected by first spraying the paper with ninhydrin (0·3 g in 100 ml *n*-butanol plus 5 ml glacial acetic acid) followed by dipping in a 1% *p*-dimethylamino-benzaldehyde solution freshly made up in acetone:conc. HCl (9:1). These areas were then cut from the chromatograms, and radioactivity determined by counting directly in scintillation fluid. Adjacent areas of each chromatogram were also counted for radioactivity.

Electron microscopy

Tissues were fixed for 2 h with 2% osmium tetroxide in 0·13 M sodium cacodylate (360 mosmol/l) at 4°C, soaked in 1% aqueous uranyl acetate at room temperature overnight, dehydrated in alcohol and embedded in Araldite. All cell suspensions were centrifuged from fetal calf serum before fixation. Ultrathin sections were stained for 3 min in saturated methanolic uranyl acetate, followed by 3 min in lead citrate (Reynolds, 1963).

RESULTS

Morphology of freshly prepared cells from guinea-pig dorsal epidermis

Histological examination of smears of cells harvested from trypsin-separated dorsal epidermis showed them to resemble closely those described by others for various species (human skin, Briggaman *et al.*, 1967; hairless mouse dorsal skin, Laerum, 1969; rat dorsal skin, Prottey, Hartop & Ferguson, 1972; and guinea-pig ear skin, Fritsch & Diem, 1972). Approximately 40–50% of the population was comprised of small, rounded cells with high nucleus to cytoplasm ratios, which were basal cells. These were well separated although a few showed distorted nuclei and ruptured plasma membranes. The remainder were comprised of stratum spinosum cells, which were larger and contained proportionately more cytoplasm, a few stratum granulosum cells, and the occasional mature squame. Cell preparations were examined by the Trypan Blue dye exclusion technique and were generally found to be 60–80% viable.

Fresh epidermal cell preparations were also examined in the electron microscope, when it was confirmed that about half of the cells were keratinocytes. Morphologically these cells closely resembled those described as guinea-pig auricular keratinocytes (Gazzolo & Pruniéras, 1969; Constable 1972). Their nuclei were large and centrally oriented, although more involuted than in basal cells *in situ*. Cytoplasmic structures tended to be arranged concentrically, with small clusters of mitochondria situated near the poles of the nuclei. There were condensed bands of randomly dispersed tonofilaments, some of which converged onto desmosomes. There was usually an outer, less dense cytoplasmic region containing scattered ribosomes, and sparse rough and smooth endoplasmic reticulum. Occasional large vesicles were seen at the periphery of the cells, with one or two desmosomes, fibres from which were continuous with the tonofilaments. Numerous long, slender and irregularly placed microvilli were seen at the cell peripheries and these infrequently showed remains of desmosomal connections in the neck region.

Development in culture of cells derived from the epidermis

The cell cultures were set up in Leighton tubes. When examined after 24 h *in vitro* many basal cells had not attached to the glass, but remained in suspension as rounded cells with shrunken pyknotic nuclei. Stratum spinosum and stratum granulosum cells and complete squames did not attach. By 48 h in culture the attached basal cells were flattened and some showed cytoplasmic granularity. An occasional strongly basophilic fusiform cell was seen. Whereas melanocytes have been routinely observed in preparations of guinea-pig ear epidermal cells (Cruickshank *et al.*, 1960; Constable, 1972), we observed no dendritic or pigmented cells in initial preparations. As we did not use phase contrast microscopy in this study, however, we cannot rule out their presence in dorsal skin preparations.

After 7 days *in vitro* the cultures contained from 20 to 50% fusiform cells, suggestive of fibroblasts (Fig. 1). These cells appeared to be metabolically active and some were bi-, tri- or multinucleated. The originally flattened epithelial-like cells were eosinophilic, some exhibiting pyknotic nuclei. On the basis of haematoxylin and eosin staining none of the epithelial-like cells appeared viable.

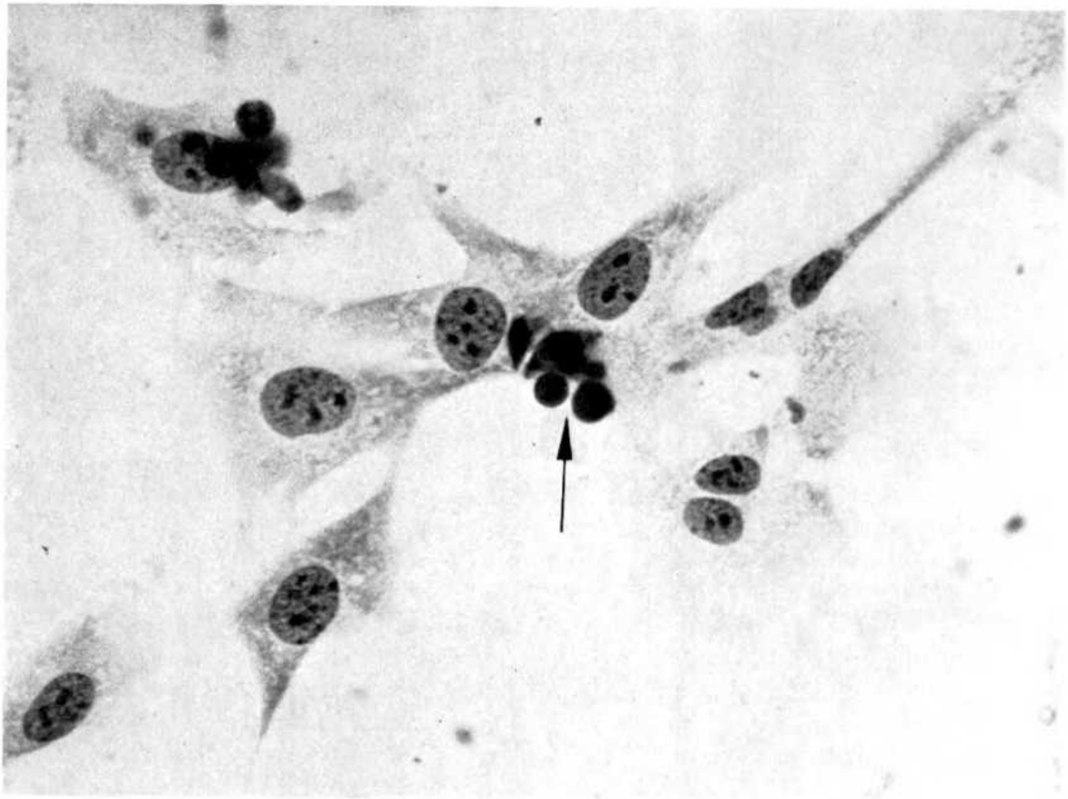

FIGURE 1. Epidermal cells after 7 days in culture. Basal cells exhibiting various stages of nuclear degeneration are shown by the arrow (H & E, × 620).

After 14 days in culture between 70–90% of the cells attached to glass were fibroblastic in appearance, being in large colonies which were linked as confluent sheets in some areas. Mitotic figures could be seen in some of the cells. Generally, by 17–20 days these cells were fully confluent and required subculturing.

Many cultures were maintained for extended periods (greater than 70 days) and it was always observed that fusiform cells persisted, often growing over each other at confluency. Fig. 2 shows a

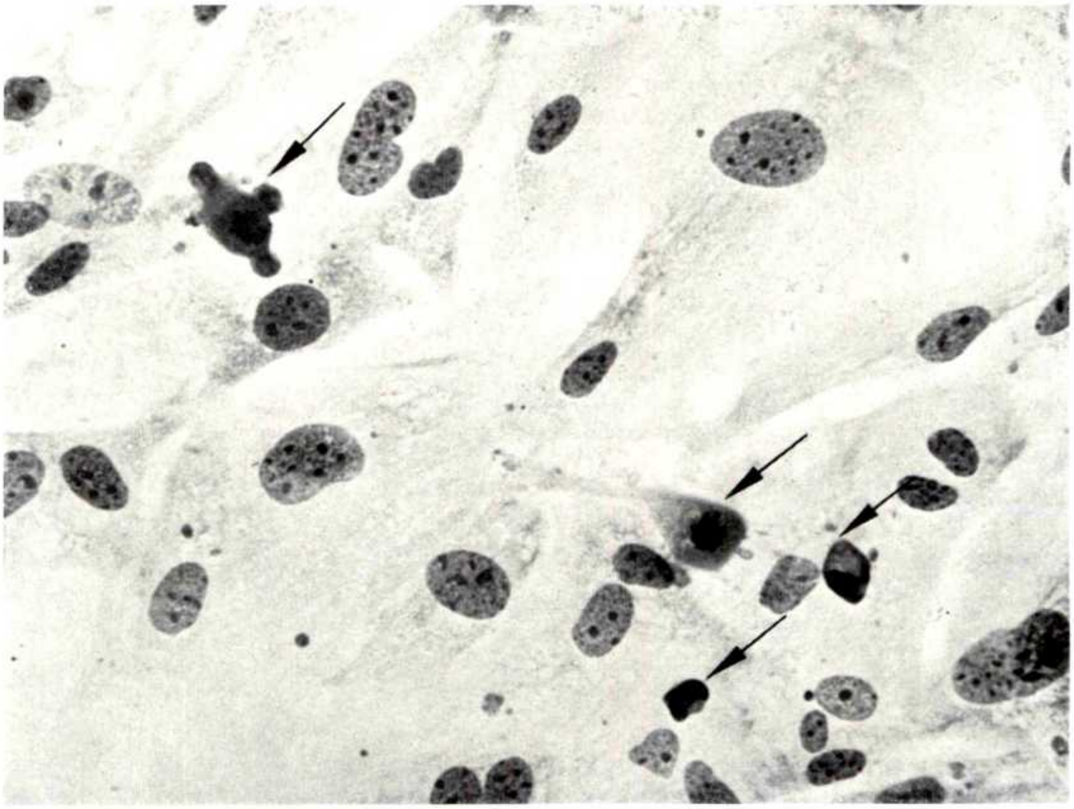

FIGURE 2. Long-term culture (60 days) of fusiform cells derived from guinea-pig keratinocyte primary cultures. The arrows show pyknotic epidermal basal cells entrapped within the network of proliferating cells (H & E, × 570).

confluent culture after 60 days and the arrows indicate pyknotic remains, presumably of original basal cells from the initial cell preparations. These cultures usually terminated by spontaneous death. One preparation was maintained for 216 days, the original culture having been subcultured fifteen times whenever confluency was reached. Thus, the average doubling time was about 2 weeks.

Ultrastructure of cultured cells derived from epidermis

Cells from 5-week-old cultures derived from epidermal preparations were examined by electron microscopy and are illustrated in Fig. 3. The figure shows cells packed closely together, presumably a feature of pelleting prior to fixation. Although about 50% were seen in groups of 2–4 cells, the isolated cells were generally not rounded as seen with freshly harvested keratinocytes. The plasma membranes of these cells were without desmosomal connections but there were many blunt irregularly surfaced protrusions from the cell surfaces (see arrows), several of these arising from one cell section accounting for 10–20% of its total cytoplasmic volume. Also, there were some dense areas of cytoplasm at or near these protuberances. The nuclei (nuc) were large and multilobed, with deep invaginations. Cytoplasmic contents were arranged roughly concentrically in a similar fashion to that found in freshly isolated uncultured basal cells. The mitochondria (mit) tended to lie around the nucleus, outside which was a region containing large spherical or stellate lipid droplets (lip). Indeed some cells (not illustrated

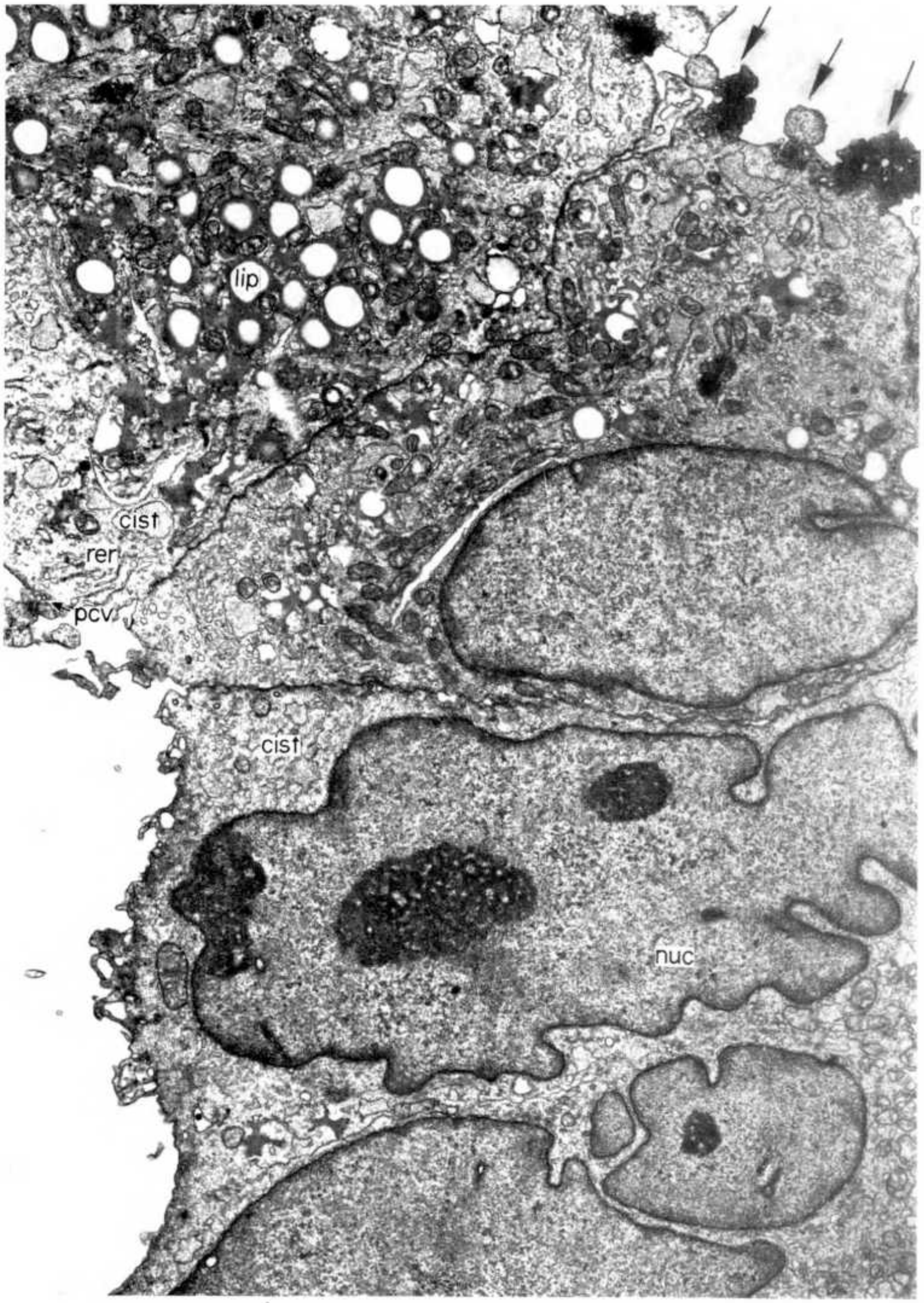

FIGURE 3. Electron micrograph of fibroblastic cells derived from epidermal preparations after culture for 5 weeks *in vitro*. Pinocytotic vesicles (pcv); lipid (lip); cytoplasmic protuberances (arrows); rough endoplasmic reticulum, (rer); and distended rough endoplasmic reticulum cisternae (cist). Note the absence of desmosomes between adjoining cells, and the nuclear invaginations (uranyl acetate and lead citrate, × 5620).

here) contained vast numbers of lipid droplets throughout the cytoplasm. The cisternae of the distended rough endoplasmic reticulum (cist) contained an homogenous pale granular matrix, similar to that of fibroblasts *in situ*. These cisternae communicated normally with neighbouring undistended rough endoplasmic reticulum (rer). A network of loosely arranged fine fibres extended outwards from regions rich in rough endoplasmic reticulum in some of the cells (not shown here). The outermost part of the cells contained only pale cytoplasm, with free ribosomes, small vesicles and smooth endoplasmic reticulum. These vesicles were often arranged near the surface and resembled pinocytotic vesicles (pcv). There was no evidence of extracellular precipitation of collagen.

Development and morphology of bone marrow fibroblasts in culture

For comparison, authentic bone marrow fibroblasts were prepared by the method of Friedenstein *et al.* (1970). This source was chosen rather than guinea-pig dermis in order to exclude the possibility of contamination by epidermal cells. The initial cell suspensions were seen to contain mainly blood cells (neutrophils) and histiocytes with only a few fusiform cells. The blood cells did not attach to glass during culture and were rinsed away in the first two changes of media. After about 3 days in culture histiocytes were in the majority, and by 7 days small colonies of fibroblasts had appeared (Fig. 4). By 15 days in culture the histiocytes had disappeared, with the fibroblasts forming large discrete colonies, which ultimately formed confluent sheets of cells after 3–4 weeks. These cells closely resembled those arising from epidermal preparations as shown in Fig. 1.

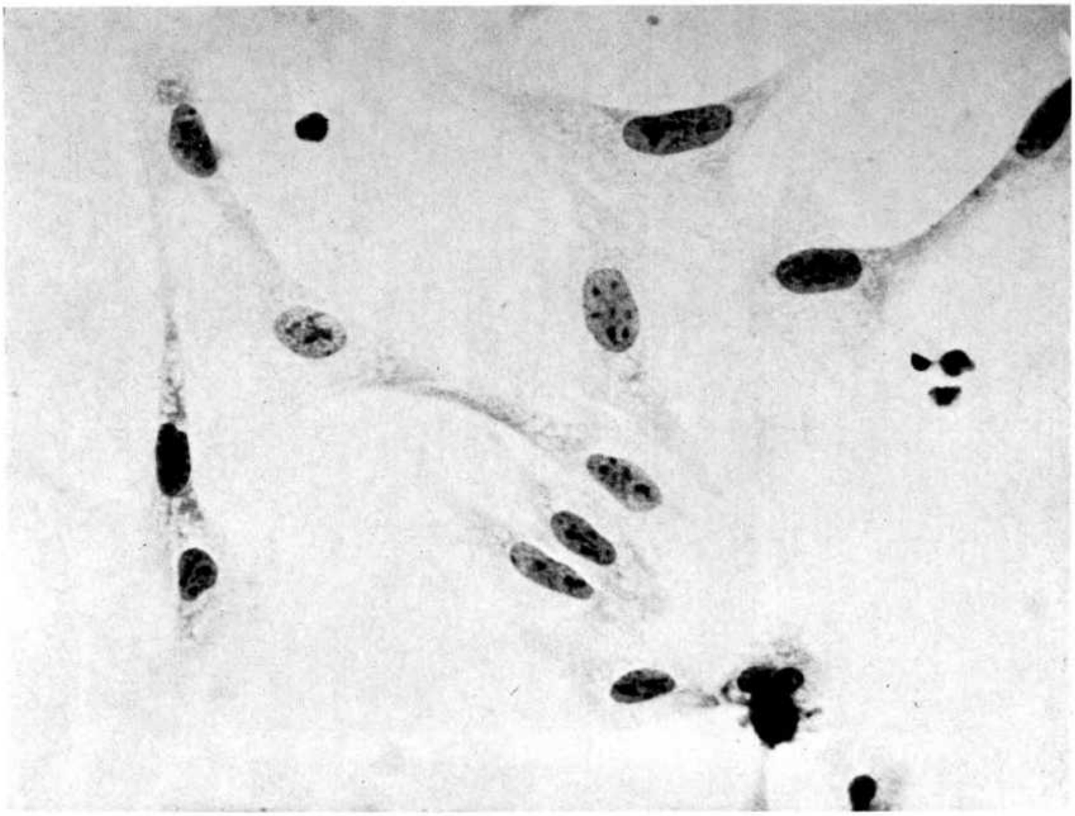

FIGURE 4. Bone marrow fibroblasts after 7 days in culture (H & E, × 560).

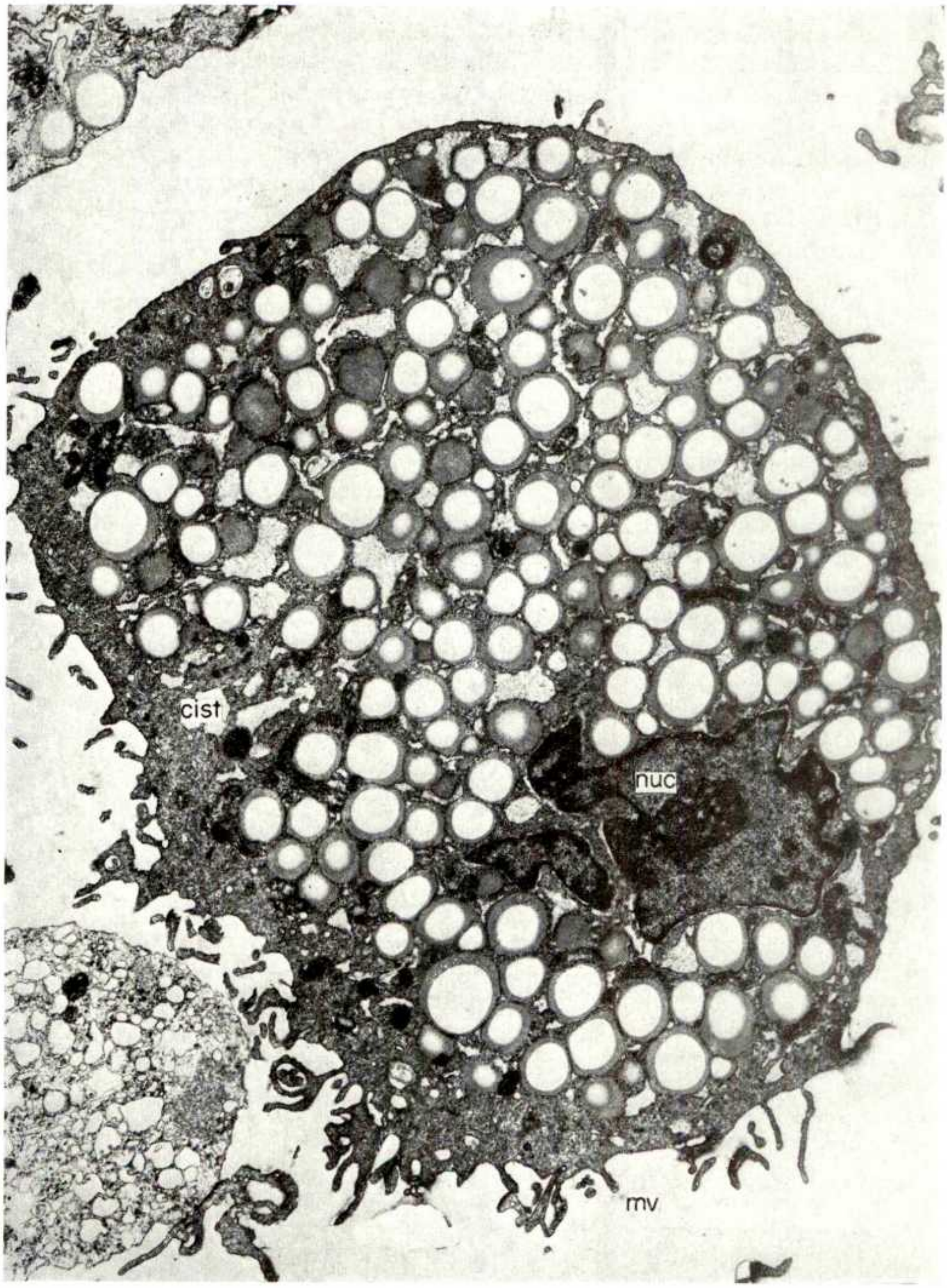

FIGURE 5. Electron micrograph of a fibroblast derived from bone marrow cultures (28 days old, subcultured after 14 days). Cisternae (cist); irregular microvilli (MV); and nucleus (nuc) (uranyl acetate and lead citrate × 5625).

These cells were examined by electron microscopy (Fig. 5) and many similarities were seen with the cells derived from long-term cultures of epidermal cell preparations (Fig. 3). Both the bone marrow fibroblasts and the skin cells exhibited large, deeply invaginated nuclei (nuc) with one (and often more) large dense nucleolus per section of nucleus. Desmosomal linkages between adjacent cells, characteristic of epidermal cells, were absent, but both cell types showed plasma membranes thrown into many irregular microvilli (mv). Small pinocytotic vesicles were seen around the cell peripheries. The rough endoplasmic reticulum (rer) was well developed with expanded cisternae in both (cist), typical of cells which actively synthesise protein, but quite unlike basal cells of the epidermis. Again, large amounts of lipid had accumulated in the fibroblasts during culture.

The ultrastructure of the bone marrow fibroblasts was very similar to that of those cells derived from long-term cultures of epidermal basal cell preparations, which in turn, resembled fibroblasts *in situ* as described by Chapman (1967), and also displayed many of the features described by Cireli (1970) for human skin fibroblasts.

Collagen synthesis in cultured cells

Fibroblasts are known to synthesize collagen *in vitro* (Green & Goldberg, 1965). This protein is specific in that it contains hydroxyproline and hydroxylysine, amino acids which are classically absent from other mammalian proteins (Adams, 1970). The ability of fibroblasts to incorporate proline into collagen and then hydroxylate a proportion of the molecules was used as a specific test for the cells in culture. In cultured fibroblasts it is known that collagen synthesis occurs only when the cells have reached confluency, and exponential growth has ceased (Green & Goldberg, 1965). The method of assaying collagen synthesis involved measurement of the degree of hydroxylation of ^{14}C-proline in collagen extracted from cultured cells. The incubation system was compiled from a consideration of recently published methods. When collagen is heated to 100°C it is converted to a water-soluble protein (gelatin), and this property was utilized for extraction of labelled collagen from the cultured cells.

Fibroblastic cultures derived from skin preparations were taken at different stages of confluency and the synthesis of collagen measured. The results are shown in Table 1. Although each culture contained 2 μCi [U-^{14}C]-proline during the 2 h incubations, absolute incorporations were quite low. Freshly harvested cells (appearing wholly as epidermal cells microscopically) incorporated 8099

TABLE 1. Collagen synthesis in cultured cells from skin

Cells	Radioactivity incorporated in collagen* (d/min)	Collagen radioactivity recovered as hydroxyproline (%)
Freshly harvested epidermal cells	8099	0.35
Skin cells approaching confluency	8535	3.69
Confluent skin cells, prior to subculture	22,087	4.36
Skin cells after subculture	67,703	0.51

Cultures contained 2 μCi [U-^{14}C]-proline, sodium ascorbate (1.0 mM), α-ketoglutarate (1·0 mM) and ferrous ammonium sulphate (0·1 mM) in a final concentration of 1·0 ml. Incubation was for 2 h at 37°C.

* Collagen extracted by 5% trichloracetic acid at 100°C.

d/min of ^{14}C-proline into collagen (i.e. the protein was solubilized by trichloracetic acid at 100°C), of which 0·35% was located in hydroxyproline. This indicated that collagen synthesis was very low in the fresh preparations, where it was due presumably to contaminating fibroblasts from the dermis. Twenty-eight-day-old cells (now wholly of fibroblastic appearance) which had been subcultured once at 14 days and consequently were now near confluency, incorporated 8535 d/min of labelled proline into collagen, but here almost 4% was hydroxylated. 28-day-old cells which had never been subcultured but which were confluent and had ceased dividing, incorporated 22,000 d/min of ^{14}C-proline into collagen, of which 4·36% was found in hydroxyproline. In similar cells which had been subcultured twice (at 14 days and at 28 days immediately before assay of collagen synthesis) only 6703 d/min were incorporated, of which only 0·5% was hydroxylated, indicating that collagen synthesis was very low following subculture.

A further experiment was performed, with increased amounts of ^{14}C-proline (10 μCi) for longer incubation times (24 h). In this case cells in 4 oz. medical flat bottles were used; these cells had reached confluency after 37 days *in vitro*, but had not been subcultured for a further 10 days prior to assay. The total amount of radioactivity incorporated into collagen was 814,568 d/min, of which 3·6% was found in hydroxyproline. For a control, 4 oz. bottle cultures of fibroblasts, derived from guinea-pig bone marrow, which were 34 days old but had not been subcultured for 14 days prior to assay, were used. Here a total of 132,042 d/min were incorporated into collagen of which 2·9% was found in hydroxyproline. These data showed that the cells derived from skin were capable of synthesizing collagen *de novo*, thus suggesting that they were true fibroblasts of mesenchymal origin. This ability was also seen to depend upon the degree of confluence of the cultures, as reported also by Green & Goldberg (1965). The extent of collagen synthesis was of the same order as that in true fibroblasts derived from bone marrow.

DISCUSSION

With guinea-pig ear keratinocytes in culture it has been reported that their initial preparation introduces minimal risk of fibroblast contamination (Cruickshank *et al.*, 1960; Pruniéras *et al.*, 1969), and so the fusiform cells observed to develop in long-term cultures are dedifferentiated keratinocytes and not fibroblasts *per se*. Recently, Regnier *et al.* (1973) have reported that two distinct types of cells may be derived from cell cultures of trypsin-separated guinea-pig auricular epidermis and dermis. These are, respectively, true keratinocytes which partially retain the specific morphology of the 'fibre-desmosome' apparatus (these cells are relatively slow to replicate *in vitro*), and true dermal fibroblasts which closely resemble bone-marrow fibroblasts and actively replicate *in vitro*. Their finding that the fibre-desmosome complexes were maintained *in vitro* is significant, as such structures are specific for the epithelium. In our long-term cultures of dorsal skin cells we found no evidence of these structures.

The results we describe for dorsal skin keratinocytes in culture have been given a different interpretation from those amply reported for the ear. Whereas we have not (and cannot, from the scope of our studies) prove unequivocally that the fusiform cells observed in long-term cultures were indeed fibroblasts and not differentiated keratinocytes, we believe this to be the case. The presence of much more follicular tissue in 200 μm thick keratotome slices of dorsal skin than in ear skin introduces a much greater risk of dermal fibroblast contamination of the primary cultures.

We believe that the light microscopic appearance of the cells in long-term cultures of guinea-pig dorsum keratinocytes is suggestive of their being fibroblasts (Figs. 1 and 2). To substantiate this we have included an illustration of guinea-pig bone marrow fibroblasts (Fig. 3), this source of fibroblasts being chosen deliberately to obviate any risk of epithelial cell contamination, and we submit that there are close similarities between the cells derived from epidermal primary cultures and authentic fibroblasts from bone marrow.

Jacquemont & Pruniéras (1969) have also discussed the possibility of dermal contamination of epithelial cell preparations; using a specific histochemical strain for connective tissue cells (naphthylamidase) these workers excluded the possibility of fibroblast contamination in guinea-pig ear preparations, but with human skin preparations, however, contamination was highly probable. In a later study of Pruniéras, leucine aminopeptidase staining was used to show that guinea-pig epithelial cell preparations contained no contaminating fibroblasts (Regnier *et al.*, 1973). We have chosen to avoid such techniques in this study, however, as in our hands we found this latter histochemical staining to be non-specific. When sections of whole guinea-pig dorsal skin were stained for leucine aminopeptidase activity, although the epidermis was negative, we observed widespread activity throughout the dermis, in the sebaceous glands, hair bulbs, dermal papillae, the erector pili and in close proximity to the capillaries, as well as in fibroblasts. Rather, we have used the criterion of collagen synthesis to corroborate the suggestion that cells surviving in long-term cultures of keratinocytes are truly fibroblasts. Whereas many cell-types may incorporate proline into proteins (including those of the epidermis) only fibroblasts have the ability to form hydroxyproline from proline in the dermis (McLennan & Karasek, 1966). There has been discussion as to whether the epidermis can also synthesize the collagen component of the basal lamina (Hay, 1964), and so collagen synthesis may not be an exclusive property of fibroblasts; however, the findings of McLennan & Karasek (1966) would not support this. As we observed quite similar levels of proline hydroxylation in cultures derived from both bone marrow and epidermis, we would suggest that the latter cells are indeed fibroblasts.

A consistent feature of our studies on the culture of epithelial cells has been their gradual disappearance from the culture vessels within the first few days. Initially they would attach to the glass walls the culture tubes, only subsequently to detach and die during which time the cultures became overgrown with cells resembling fibroblasts. It is well known that organ culture of isolated epidermis is unsuccessful without the presence of dermis or dermal components (Dodson, 1967; Briggaman & Wheeler, 1968). This has been shown by some to be true for isolated epidermal cells also, Ingemansson-Nordqvist, Kiistala & Rorsman (1967) showed that growth of human epidermal cells was greatly prolonged on films of collagen gel compared with glass substratum, and Karasek & Charlton (1971) reported that, although rabbit keratinocytes had only a limited lifetime *in vitro* of about 7 days, when collagen gel was the substrate and if a growth medium which had previously been exposed to growing fibroblasts (a fibroblast-conditioned medium) was used, then the keratinocytes survived for up to 3 weeks and demonstrated horizontal and vertical growth.

We have also found that collagen substratum greatly enhances the metabolic activity of primary cultures of guinea-pig dorsum keratinocytes (Prottey & Ferguson, 1974) and it is likely that correct choice of culture conditions would facilitate their prolonged survival *in vitro*. Nevertheless, the problem of contamination by dermal fibroblasts still remains when one chooses the dorsal skin of guinea-pigs as a source of keratinocytes for culture experiments.

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The metabolic activity of primary cultures of guinea-pig dorsal skin cells

C. PROTTEY AND T. F. M. FERGUSON

Unilever Research, Colworth Welwyn Laboratory,
Colworth House, Sharnbrook, Bedford MK44 1LQ

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SUMMARY

Freshly harvested epidermal cells from the dorsal skin of albino guinea-pigs have been cultured for several days on glass slides and on collagen films within Leighton tubes. Routinely it has been observed that the keratinocytes which initially attach to the substratum rapidly detach again during the first 6 days and the presence of collagen does not influence their overall survival. However, the keratinocytes cultured on collagen films exhibit greatly enhanced cellular metabolism, in particular, dehydrogenase activity, DNA synthesis and phospholipid turnover. These findings support those published by others that collagen (and, presumably, other components of dermis) greatly influences the epidermis *in vitro*.

In an accompanying paper (Prottey, Tovell & Ferguson, 1974) we have described how epidermal keratinocytes prepared from the dorsal skin of guinea-pigs possess but limited viability in primary culture. After only about 6 days *in vitro* these cells detach from the walls of glass culture vessels and die, and in most cases the vessels subsequently become overgrown with fibroblasts originating from contamination of the initial preparations. This situation is quite unlike that seen with similar preparations derived from guinea-pig ear, in which it has been amply reported that keratinocytes may survive for many weeks, and usually with dedifferentiation of their original morphology (Cruickshank, Cooper & Hooper, 1960; Constable, 1972; Regnier, Delescluse & Pruniéras, 1973).

This limited viability and problem of contamination of dorsal skin keratinocytes were not altogether unexpected as there are reports that the correct choice of substrata for culture is important for the maintenance of skin epithelial cells *in vitro*. For example, Ingemansson-Nordqvist, Kiistala & Rorsman (1967) showed that human keratinocytes survived for only 3–4 days on glass, whereas on rat collagen films their survival was extended to 4–6 weeks. Similarly, Karasek & Charlton (1971) have shown that rabbit keratinocytes exhibited enhanced growth on films of rabbit collagen. Indeed, the strong affinity of keratinocytes for collagen has been used as a specific means of separating germinative epidermal basal cells from non-germinative (differentiated) cells (Moore & Karasek, 1971; Prottey & Hartop, 1973). Also, it is known that the maintenance of intact epidermis in organ culture is unsuccessful without the presence of dermis or components of the dermis (Dodson, 1967; Briggaman

& Wheeler, 1968, 1971). As collagen clearly enhances the viability of primary cultures of keratinocytes, we have examined certain biochemical parameters in these cells on both collagen films and glass in order to evaluate whether cellular metabolism is affected by the type of substratum used.

MATERIAL AND METHOD

All methods of preparation and culture of guinea pig dorsum keratinocytes have been described in the accompanying paper (Prottey *et al.*, 1974).

Collagen was extracted from rabbit skin exactly as described by Karasek & Charlton (1971). Solutions of 3 mg/ml were used for preparation of films by adding six drops to Leighton tubes and then exposing to the vapour from ammonium hydroxide solution sp.gr 0.880 overnight. It was impossible to ensure that the collagen solutions were sterile; however, after exposure to ammonia we never observed growth of micro-organisms. The collagen films thus produced were rather translucent, which severely hampered microscopic examination of attached cells.

Radiochemicals were purchased from the Radiochemical Centre, Amersham, Bucks.

Dehydrogenase activity in primary cultures of guinea-pig dorsal skin cells

Keratinocytes were prepared from male guinea-pigs and suspended in Eagle's medium containing 10% fetal bovine serum and 10% guinea-pig serum at a density of $2{\cdot}87 \times 10^6$ cells/ml. By using dye exclusion (Wiepjes & Prop, 1970) their viability was assessed to be 87%. Portions of 1·0 ml were added to a series of glass Leighton tubes, some containing films of collagen, which were then gassed with 5% CO_2 in air and incubated at 37°C for several days. Dehydrogenase was assayed according to the method described by Marks & James (1959) in initial cell suspensions and after culture for 1, 2, 5 and 6 days. In those tubes containing collagen films, culture was discontinued after the sixth day due to shrinkage and detachment of the films from the vessel walls, but in the cultures on glass alone measurements were also made after 14 and 21 days.

Incorporation of $[^{14}C]$ *thymidine and* $[^{32}P]$ *orthophosphate into keratinocytes* in vitro

A series of Leighton tubes containing glass cover slips were inoculated with 1·0 ml of a suspension of freshly prepared guinea-pig epidermal cells ($3{\cdot}24 \times 10^6$ cells/ml). In some of the tubes the cover slips were coated with films of rabbit skin collagen. The cells were then incubated at 37°C and every 24 h the medium was replaced by decantation (Eagle's medium + 10% fetal bovine serum and 10% guinea-pig serum). Pairs of tubes were taken to which was added either $[2\text{-}^{14}C]$ thymidine (2·5 μCi, carrier free) or $[^{32}P]$ orthophosphate (25 μCi, carrier free) and then incubated for 24 h at 37°C, at which time the cells were killed by adding trichloracetic. The radioactivity incorporated into DNA and phospholipids was determined as previously described (for DNA, Ferguson & Prottey, 1974; for phospholipids, Prottey, Hartop & Ferguson, 1972). Pairs of tubes were taken at successive 24 h periods, thus metabolic activity in the cells was examined during the periods 0–24 h, 25–48 h, 49–72 h and so on, for 7 days of culture (6 days only for the phosphorus experiment).

RESULTS

Dehydrogenase activity in keratinocytes cultured on glass and collagen substrata

The results are shown in Fig. 1. Keratinocytes maintained on glass showed a rapid decline in dehydrogenase activity within the first 24 h of culture which remained at virtually zero through 6 days. Light microscopy of these cultures showed that following the initial attachment of rounded and flattened basal cells during the first 24 h there was a subsequent rapid detachment such that by the sixth day only an occasional keratinocyte was seen. At the sixth day, however, numerous fusiform cells began to

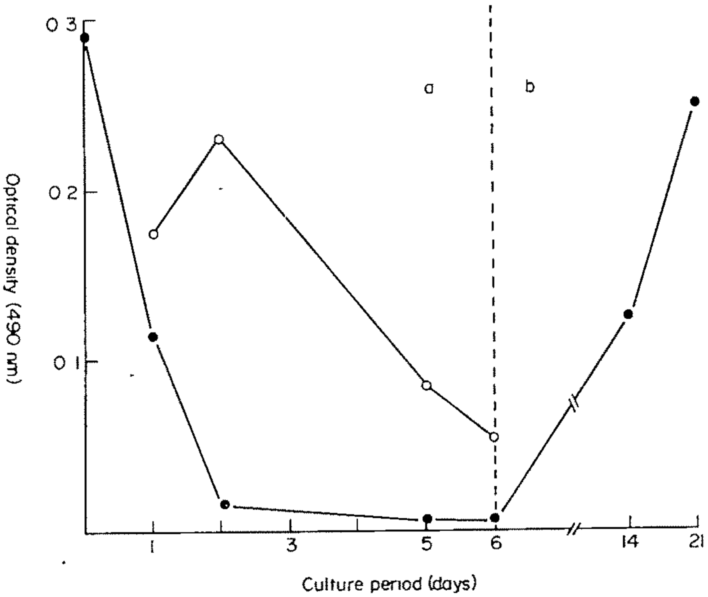

FIGURE 1. Dehydrogenase activity in keratinocytes cultured on glass (●——●) and collagen film (○——○). (a) keratinocytes present; (b) fibroblasts present.

appear and their numbers rapidly increased; by the fourteenth day the glass slides of the Leighton tubes were largely covered by colonies of these cells, and at 21 days there were confluent sheets. These cells were assumed to be fibroblasts derived from dermal contamination of the original cell preparations, as described elsewhere (Prottey *et al.*, 1974).

With keratinocytes maintained on collagen films, however, a consistently higher level of dehydrogenase activity was detected throughout the 6 days in culture. Microscopic examination of the cells attached to the collagen films suggested that their viability was enhanced; a rough estimate of the cells remaining attached suggested that some keratinocytes remained attached during the 6 days, and fewer of these, relative to those on glass, showed shrunken and pyknotic nuclei and rounded cell peripheries. However, even on collagen there was massive detachment at later times relative to those which originally attached during the first 24 h. In the cultures on collagen we also observed the appearance of fibroblasts at about 6 days, but a major drawback of this technique was that the films gradually contracted and curled up to such a degree that by the sixth day many had detached from the glass completely. Therefore, dehydrogenase estimations were not continued after 6 days in these cases.

DNA synthesis in keratinocytes cultured on glass and collagen substrata

The incorporation into DNA of [^{14}C] thymidine added to the culture medium was measured and the results are shown in Table 1. In cultures on glass the level of DNA labelling was uniformly low, being highest after 4 days (i.e. between 72–96 h *in vitro*), but by the seventh day this was zero. On collagen films, however, the labelling was generally higher and maximal during the second day in culture, but again, as seen in the dehydrogenase experiment, metabolic activity gradually decreased with time. Again, microscopic examination of the cultures on both glass and collagen revealed a progressive detachment of those cells which originally attached, many of which had shrunken nuclei. As there was no sign of mitotic figures in any cells remaining attached it was assumed that the cells only synthesised DNA once during the experiment; thus, by summing the total conversion of labelled thymidine to DNA it seems that five times the number of cells on collagen substratum synthesized

DNA compared with those on glass. After the sixth day of the experiment fusiform cells (i.e. fibroblasts) were seen in both types of culture.

TABLE 1. DNA synthesis in keratinocytes cultured on glass and on collagen*

Period of radioactive pulse (h)		Total radioactivity recovered in DNA (dpm)	
		On glass	On collagen
0–24		147	378
25–48		594	3979
49–72		686	2703
73–96		1616	1697
97–120		860	760
121–144		215	683
145–168		0	471
	Totals	4118	10671

* Each incubation contained $3{\cdot}24 \times 10^6$ cells and $2{\cdot}5$ μCi [2-^{14}C] thymidine in a total volume of 1·0 ml.

Phospholipid synthesis in keratinocytes cultured on glass and collagen substrata

The turnover of phospholipids in cells was examined by measuring the uptake into total cellular lipids of [^{32}P] orthophosphate added to the culture medium. Table 2 shows that with the keratinocytes cultured on glass there was very little phospholipid labelling except after 6 days *in vitro*, when light microscopy showed that none of the original keratinocytes remained attached, but numerous fibroblasts were evident.

TABLE 2. Phospholipid turnover in keratinocytes cultured on glass and on collagen*

Period of radioactive pulse (h)	Phospholipid radioactivity (dpm)	
	On glass	On collagen
0–24	1228	191,200
25–48	2345	404,700
49–72	0	349,200
73–96	0	352,800
97–120	585	371,800
121–144	18,900	397,700

* Each incubation contained $3{\cdot}24 \times 10^6$ cells and 25 μCi [^{32}P] orthophosphate in a total volume of 1·0 ml.

In those cells maintained on collagen, however, massive radioactivity was incorporated into phospholipids, and was sustained throughout the period of the experiment. Although this suggested a sustained enhanced metabolism of the keratinocytes attached to the collagen, we still observed a progressive decline in the number of cells attached to collagen during the second, through to the sixth day.

DISCUSSION

The data described here resemble in some ways those reported by others on the longevity of keratinocytes from the skin of other species. On glass substratum, human keratinocytes (Ingemansson-

Nordqvist *et al.*, 1967) and rabbit keratinocytes (Karasek & Charlton, 1971) rapidly degenerate, but when cultured on collagen films their survival is greatly extended. We have been unable to obtain extended survival times *in vitro* with guinea-pig dorsum keratinocytes for longer than 1 week. It is well known that intact epidermis (devoid of any dermis) cannot survive *in vitro* without the presence of either dermis, dermal extracts or some components of the dermis (Dodson, 1967; Briggaman & Wheeler, 1968, 1971), and so the effect shown by collagen here with isolated epidermal cells may be similar. Equally, however, there are many reports of the long-term survival *in vitro* of isolated keratinocytes on glass (Cruickshank *et al*; 1960; Karasek, 1966; Briggaman *et al.*, 1967) and so it is surprising that we were routinely unsuccessful at maintaining guinea-pig dorsal skin keratinocytes *in vitro* for longer than a week. It may be that during their isolation, which involved controlled trypsinization, the cells were damaged by the trypsin, as has been shown to occur by Jensen & Mottet (1970) with the isolation of intact epidermis by this technique. In the many published papers on the preparation of keratinocytes using trypsin it is probable that there are many different individual procedures (length of digestion, concentration of the protease). It is also known that there are wide variations in protease activity between different batches of trypsin (Pine *et al.*, 1969), and so we may have routinely harvested irreparably damaged cells which had only limited viability.

Despite this possibility, it is clear from this study that the presence of collagen greatly influences the vitality of basal cells. The property of adhesion to collagen films possessed by germinative keratinocytes (i.e. those which have not yet differentiated) allows one to separate them from differentiated cells. Moore & Karasek (1971) used this technique to demonstrate that the chemical composition of basal cells was different from differentiated cells, and also we have used the technique to distinguish between lipid metabolism in germinative and in differentiated cells of rat epidermis (Prottey & Hartop, 1973).

Collagen may not be the best substratum for maintaining epithelial vitality *in vitro*, indeed, Briggaman & Wheeler (1971) have shown that it is inadequate for maintaining sheets of intact epidermis, whereas powdered dermis is very effective. In metabolic studies on isolated epidermal cells *in vitro* our results suggest that use of guinea-pig dorsal skin as the source results in cells of only limited viability; however, collagen as a substratum may result in demonstration of biochemical activity much closer to that in the intact epidermis *in vivo*.

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British Journal of Dermatology (1974) **91**, 687.

Hereditary hypotrichosis simplex of the scalp

EVIDENCE FOR AUTOSOMAL DOMINANT INHERITANCE

JAIME TORIBIO AND PEDRO A.QUIÑONES

Department of Dermatology (Head: Professor P.A.Quiñones)
Faculty of Medicine, University of Valladolid, Spain

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SUMMARY

A peculiar variety of hereditary hypotrichosis is described. The abnormality has affected a large family over the course of eight generations. This genealogy is the fullest so far known of hereditary hypotrichosis.

The condition is a hypotrichosis limited to the scalp, which first manifests itself in the early school-years. Afterwards the loss of scalp hair gradually progresses to almost total alopecia. This terminal stage is reached between the ages of 20 and 25 years, and the condition affects males and females in a similar way. From the genetic point of view, the abnormality is transmitted with an autosomal dominant mode of inheritance with full penetrance.

Hypotrichosis is a relatively common feature of a number of complex hereditary syndromes, in association with other anomalies or ectodermal defects, but simple hereditary hypotrichoses (that is, not associated with any other abnormalities) are very infrequent; even rarer are those limited exclusively to the scalp.

The present paper deals with a peculiar form of simple hereditary hypotrichosis of the scalp, affecting a large kindred in the village of Villafranca de Duero, in the province of Valladolid, as well as a smaller offshoot of that family in Fuentelapeña, in the province of Zamora (Spain).

MATERIAL AND METHODS

The full genealogy of the affected family group was established by the research we conducted in the church records of Villafranca de Duero, as well as by important data communicated to us by the oldest surviving members of the family (fifth generation) who were able to provide very precise information about their grandparents (third generation).

The clinical investigation was carried out on a total of thirty patients, eight of whom were hospitalized for more thorough study in the Department of Dermatology of the University of Valladolid.

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* A preliminary report was read at the 7th Annual Convention of the Chair of Dermatology, Complutense University (Professor J. Gómez Orbaneja), Madrid, 8–9 April, 1972.

These eight patients, of both sexes, and of ages ranging from 9 to 47, typified the different phases of the evolution of this form of hypotrichosis.

Extensive investigations were performed on these eight patients; including routine blood counts, serum calcium and phosphorus, alkaline phosphatase, electrolytes, plasma lipids, creatinine, serum proteins, immunoelectrophoresis, chromatography of aminoacids and karyotype. Urinalysis included standard tests, sodium, potassium, calcium, phosphorus, creatinine and creatinine clearance and chromatography of aminoacids. Saliva was examined for sodium, potassium and chloride. Dermatoglyphics, electroencephalograms and skeletal X-rays were also performed.

Samples of hair were taken by clamping a small piece of the forelock with a Pean forceps, the ends of which were covered with rubber. The hairs so removed were used only for morphological examination by light and scanning electron microscopy, since they were considered inadequate for an analysis of the hair-cycle. In fact this procedure leaves out of account the vellus-type follicles which produce no visible hairs.

The density of the hair population and the cyclical activity of the hair follicles were determined by the examination of biopsy specimens. For this purpose biopsies of the scalp were obtained from three patients, who were at different stages in the evolution of the hypotrichosis. These biopsy specimens were embedded in paraffin wax and serially sectioned at 6 μm. Seventy-five serial sections from each piece were distributed on thirteen numbered slides, and stained with haematoxylin-eosin, alcian blue-PAS-haematoxylin, and orcein. The serial sections were studied in order, counting the hair follicles and at the same time classifying them according to the different phases of the hair-cycle.

RESULTS

Inheritance

The results of the genealogical research are set out in detail in the abbreviated pedigree of the involved family (Fig. 1). Because of the large number of individuals in this family, we have not included the descendants, invariably normal, of unaffected couples.

All members of this pedigree can be traced to the offspring of the couple in generation I: Pedro G.P., born in February 1754, who died on 14 June 1816, and Isabel P.P., born in April 1758, who died on 7 November 1819. The kindred comprises a total of 213 individuals (not counting the descendants of unaffected couples), spread over eight generations. Of these 213 persons, 113 are males and 100 females. The total of affected members is seventy-eight, of which thirty-eight are males and forty females. In addition, among the 213 individuals who make up this family, there figures a group of seventeen persons whose extreme youth makes it impossible to determine whether or not they are affected.

In the pedigree the most outstanding facts to be observed are the following. There is no instance of marriage between two affected individuals. The abnormality appears in every generation, without exception. The hypotrichosis is transmitted by an affected person to the children, on average 50% of them. No unaffected individual has ever transmitted the trait. The abnormality involves and is transmitted by both sexes equally.

Clinical features

The history and evolution of the condition is virtually the same in every case, and the final result of the process is likewise identical in all. There are no partial forms, and it is a very important point that the condition affects males and females in a similar way.

The affected children are quite normal at birth and in the first years of life. The condition begins insidiously between the ages of 5 and 12 years. Within this period, a retardation of the growth of the

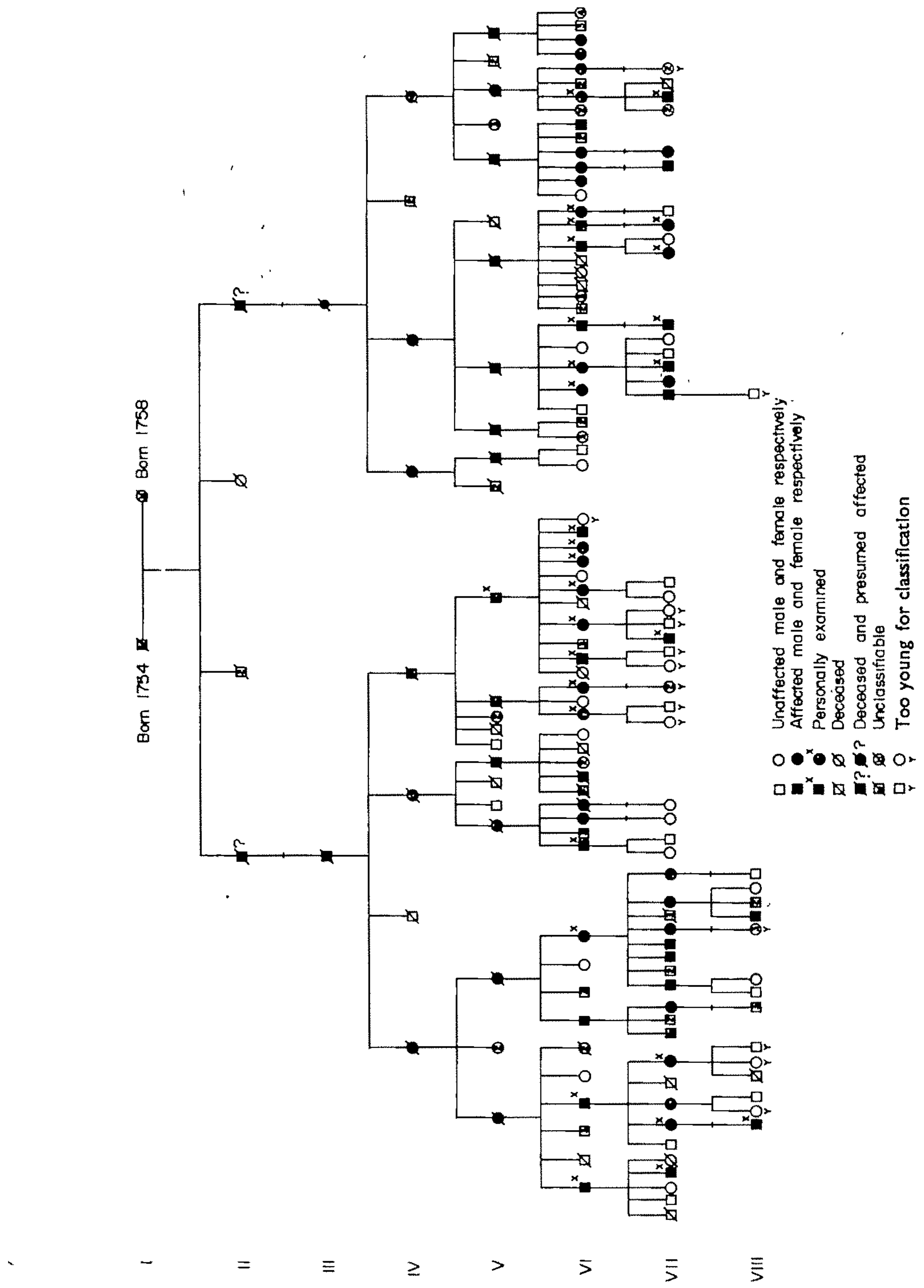
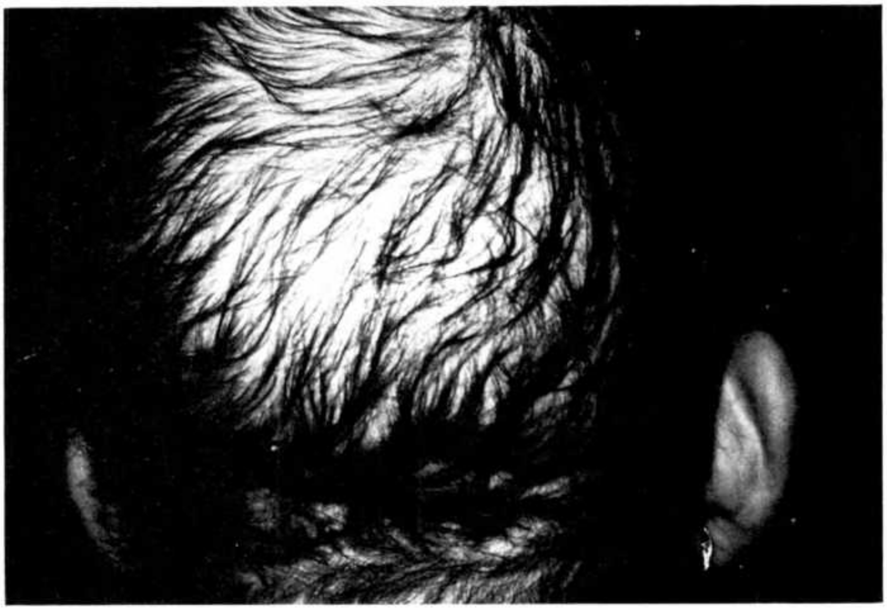
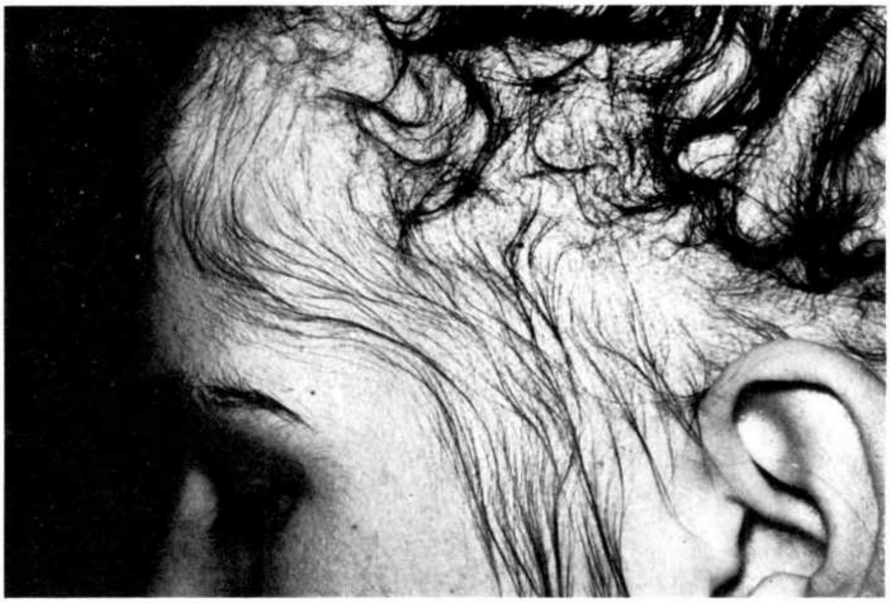

Born 1754
Born 1758
I
II
III
IV
V
VI
VII
VIII
□ ○ Unaffected male and female respectively
■ ● Affected male and female respectively
■ ● Personally examined
Deceased
? ? Deceased and presumed affected
Unclassifiable
□ ○ Too young for classification

FIGURE 1. Abbreviated pedigree of the involved family.





FIGURES 2 and 3. Middle stage in the development in the condition in a female, 18 years old.

hair is observed, together with a slow and diffuse hair loss. In these early stages the hair begins to appear noticeably thinner all over the scalp at the same time. A few years later, in the intermediate stage of the evolution of the process, the patients show a severe hypotrichosis (Figs. 2 and 3) without recession of the anterior hair line.

Between the ages of 20 and 25 years, all the affected individuals, male and female, reach the terminal stage, in which there survive on the scalp only a few sparse, fine, short hairs, of the same colour as the hair they possessed earlier (Fig. 4). The eyebrows and eyelashes are never affected, and the beard and chest hair of the males and the axillary and pubic hair of both sexes develop and persist quite normally.

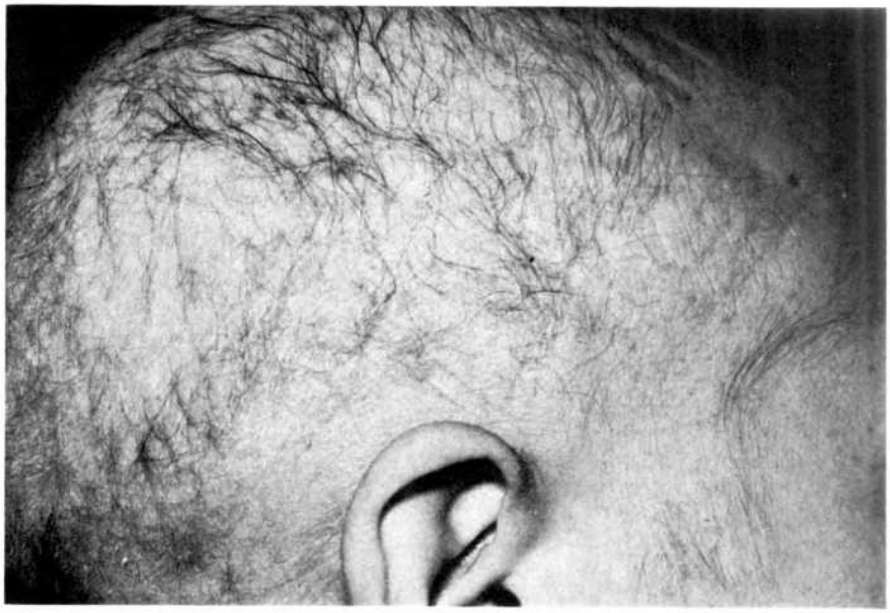


FIGURE 4. Terminal stage in a female, 32 years old.

The tegument of the scalp looks normal. In some places the follicular orifices show a slight prominence, without any manifestation of keratosis pilaris.

In the hair itself there is no macroscopic change whatsoever. There are no associated abnormalities; the nails and teeth are quite normal. To sum up, therefore, these individuals are entirely normal, except for the hypotrichosis of the scalp.

Mentally they are also normal, and there is no effect on their behaviour and attitude other than what one would expect in the face of a problem which, through so many generations, has afflicted them and their families.

Laboratory studies

All the tests performed in the laboratory on blood, urine and saliva gave normal results. The radiography of the bones, electroencephalograms, karyotypes and dermatoglyphics were likewise normal.

Microscopic and histopathological findings

The hairs showed no detectable alteration under the light microscope. Scanning electron microscopy of hairs of early hypotrichosis revealed circular shafts with normal imbricated scales (Fig. 5). In advanced hypotrichosis the hairs showed focal areas of defective cuticular structure (Fig. 6).

In the histological sections, no abnormality could be found in the epidermis, dermis or hypodermis, nor any qualitative change in the cutaneous appendages in any of the different stages of the hypotrichosis.

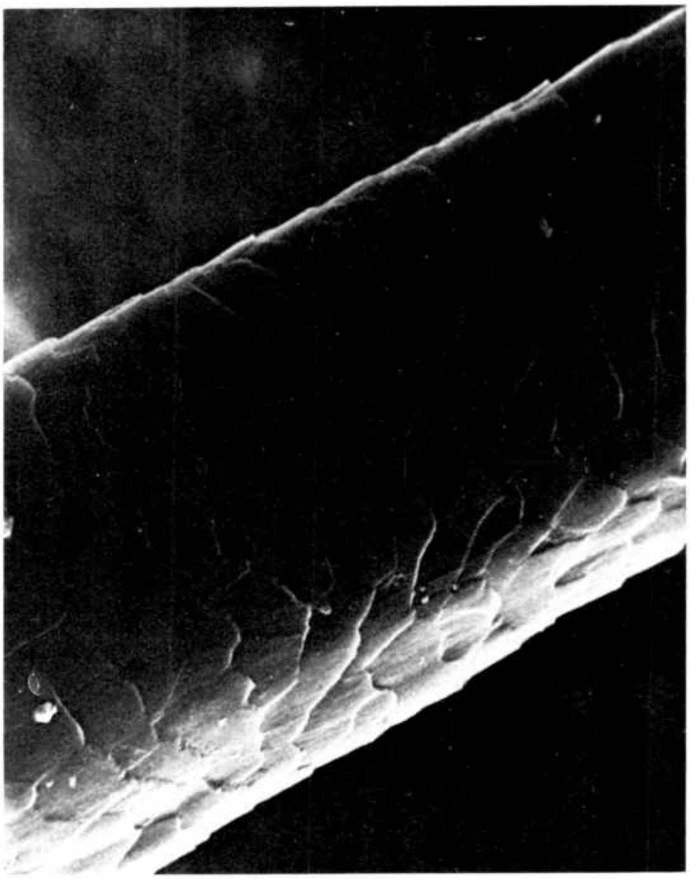


FIGURE 5. Scanning electron micrograph of hair of early hypotrichosis, showing circular shaft with normal imbricated scales (× 1250).

The results of total and differential counts of the follicles are summarized in Table 1.

From these results the following facts may be emphasized. The number of follicles is normal in all three stages of the hypotrichosis (bearing in mind the reduced length of biopsy specimens from the terminal stage). In the early stage of the hypotrichosis there are numerous follicles in the phase of developed anagen (Fig. 7). These developed anagen hairs disappear in later stages, while the number of follicles in the phase of initial or intermediate anagen increases. Catagen and telogen hairs also disappear in the terminal stage. In the middle and terminal stages the buds of the follicles in the phase of initial anagen (Fig. 8) spring from follicles of very small diameter in telogen; this seems to indicate that they have entered catagen from an intermediate anagen, which is concordant with the absence of follicles in developed anagen in the later stages of the condition.

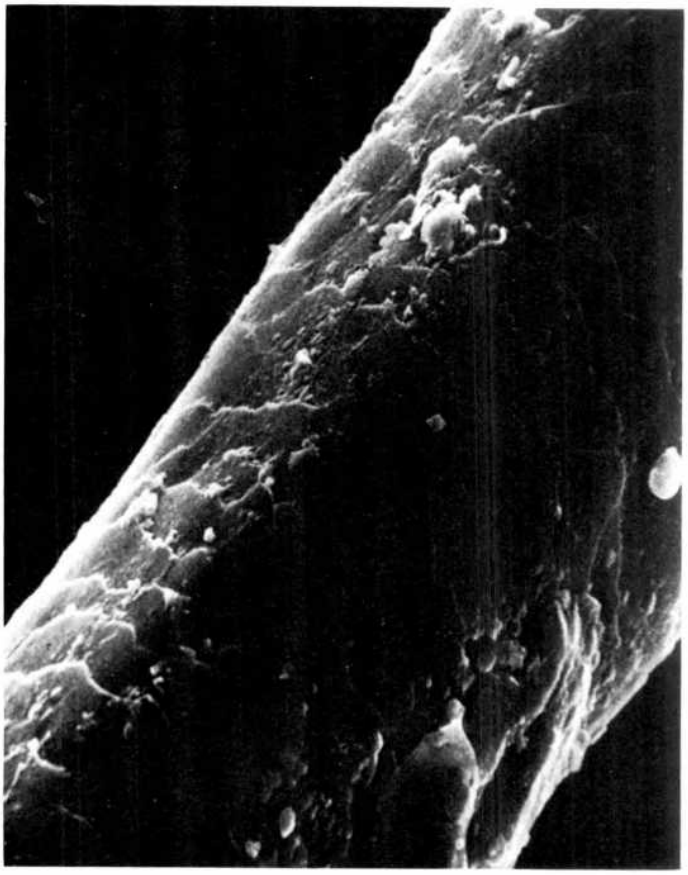


FIGURE 6. Later stage of condition, showing focal areas of defective cuticular structure (× 1250).

DISCUSSION

Within the group of hereditary hypotrichoses several clinically distinct conditions, related to different modes of inheritance, have been recognized. Hereditary hypotrichosis as an isolated disorder is very rare, and especially infrequent when limited to the scalp.

Cockayne (1933) mentioned as autosomal dominant hypotrichoses affecting exclusively the scalp, only those cases described by Ledermann (1896) and Petersen (1915). In 1896 Ledermann presented two cases, a father and son, affected by hypotrichosis which was limited to the scalp, but in which the alopecia of the father was present at birth. Petersen (1915) described a family of three generations with fourteen members, of whom nine were affected. His cases are those most similar to ours, but with

TABLE I.

Stage of the hypotrichosis Length of biopsy specimens	Early 14 mm	Middle 15 mm	Terminal 8 mm
Initial anagen	3	13	13
Intermediate anagen	11	16	11
Developed anagen	18	1	0
Catagen	4	1	0
Telogen	2	4	0
Total	38	35	24

some differences. In Petersen's cases there occurred, around the age of 4 or 5 years, a loss of hair which was replaced by shorter, sparser, and lighter-coloured hair. The hair which persisted to the age of between 16 and 20 years was colourless.

The hypotrichosis described by Jeanselme and Rime (1924) spanned five generations, with a total of thirty-five individuals, of whom fourteen were affected. Its transmission was clearly autosomal dominant, but it was not limited to the scalp, also affecting the eyebrows and eyelashes and accompanied by scanty development of axillary and pubic hair.

The hypotrichosis of Marie Unna (1925) is one of the better established prototypes, through its full genealogical documentation. Ludwig (1953) and Borelli (1954) later added other cases belonging to the same family. It also is an autosomal dominant hypotrichosis but it is not limited to the scalp, and scalp hair is sparse or absent at birth. During early childhood coarse hair grows which becomes gradu-

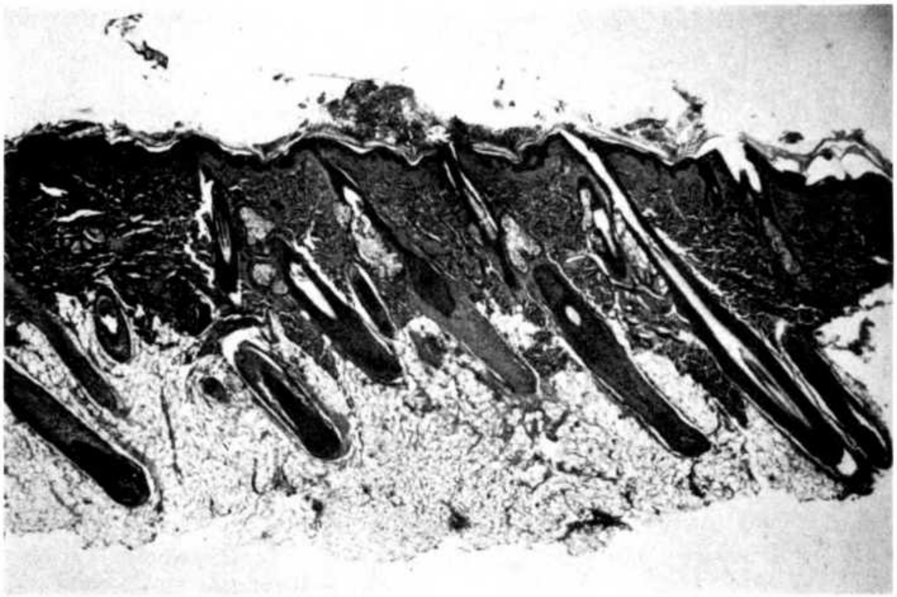


FIGURE 7. Early stage, the section shows numerous follicles in the phase of developed anagen. (H & E × 40).

ally sparse at puberty. This hypotrichosis shows a structural defect of the hair shaft, and its terminal stage simulates male pattern alopecia (Stevanovic, 1970).

Pajtas (1950) described a kindred of 147 members of whom twenty-two were affected in four generations. This also is inherited as an autosomal dominant trait, but it is congenital and generalized.

The condition reported here is a hereditary hypotrichosis limited to the scalp, and transmitted with an autosomal dominant mode of inheritance with full penetrance. The genealogical tree of the family provides a perfect example of this pattern of transmission. The trait appears in each generation, affecting males and females in an identical way. Unaffected members have never transmitted the condition, and the proportion of normal to affected children produced by affected individuals averages 50%.

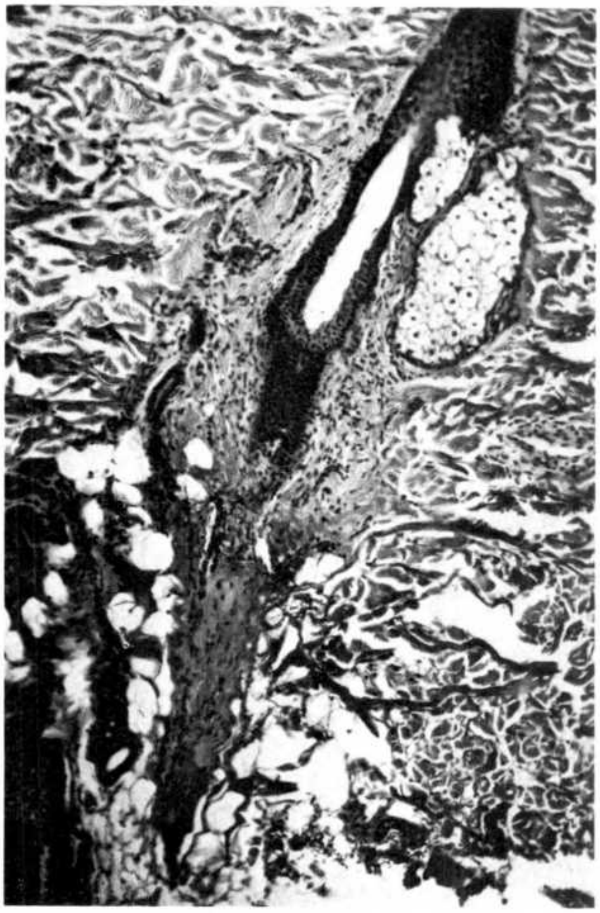


FIGURE 8. Middle stage; section showing initial anagen phase (H & E × 100).

The most interesting clinical features of this hypotrichosis are the following. The affected children are quite normal at birth and in the first years of life. The hypotrichosis appears in the primary-school years and gradually progresses involving only the scalp, without any other associated abnormality.

We conclude, therefore, that the hypotrichosis we have described here is a peculiar variety of autosomal dominant hypotrichosis, different from those hitherto reported.

The histopathological features and the results of total and differential counts of the follicles on serial sections appear to suggest that this hypotrichosis is due to the fact that the follicles gradually reduce the duration of their cycles. The duration of activity of follicles, or anagen, becomes so short that it does not even allow the hair to show through the follicular pore, in the same way as occurs in the vellus follicles of the forehead or the nose.

ACKNOWLEDGMENTS

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The pathogenic status of yeasts infecting ulcerated legs

R.J.SMITH, M.P.ENGLISH AND R.P.WARIN
Departments of Dermatology and Pathology, United Bristol Hospitals

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SUMMARY

Seventeen patients with common venous ulcers, from which Candida species were isolated, were treated with nystatin incorporated into an ointment applied once per week before the application of compressive bandages. In half the patients the regime was used for 1 month, followed by a month with the same treatment but without nystatin; this procedure was reversed in the remainder of the patients.

Regular mycological examination showed that the yeast was eradicated in all the cases when nystatin was used, but the clinical response was not closely related to the disappearance of the yeast. Sixteen patients were then followed up for a year, or until the ulcers had healed, and were treated with nystatin for as long as the yeast was shown to be present. This treatment did not have any obvious clinical effect on the rate of healing.

In a previous report in this Journal (English, Smith & Harman, 1971) it was shown that some common venous leg ulcers were infected by fungi. *Candida parapsilosis*, or occasionally other yeasts, were isolated from thirty-five out of fifty-six ulcerated legs. The yeasts always occurred in the macerated skin surrounding the ulcers, but were sometimes present in swabs from the ulcers themselves as well.

The yeasts tended to disappear at, or around, the time the ulcer healed, but whether this disappearance played any significant part in the healing of the ulcer, was doubtful. The work reported in this paper is an attempt to discover whether, by eliminating the fungus, the ulcers heal more readily.

Preliminary trial

Eight patients with ulcers known to be infected by yeasts were treated for 4 weeks with nystatin ointment, applied weekly when their occlusive bandages were changed. Yeasts were eliminated in all patients. A more detailed study was therefore attempted.

Trial 1

Patients with ulcerated legs attending a routine out-patient clinic had swabs taken from their ulcers, and scrapings from the surrounding skin. These were examined in the laboratory by the methods described by English *et al.* (1971).

Of the seventeen patients with yeast infection of their ulcers, thirteen were infected by *C. parapsilosis*, two by *C. guilliermondii*, one by *C. albicans*, one by *Torulopsis haemulonii* (English & Atkinson, 1974) and one by *C. parapsilosis* with *Fusarium oxysporum*. All the yeasts had been found to be sensitive to nystatin *in vivo*, therefore no distinction is made between them in this report.

The seventeen patients were all treated with the usual compressive bandages consisting of clioquinol (Vioform) impregnated occlusive bandages (Quinaband, Seton Products Ltd) with an elasticated diachylon bandage on top (Lestreflex, Seton Products Ltd). The dressings were changed at weekly intervals. Before the bandages were applied all ulcers were treated with an ointment containing polymyxin-B sulphate and zinc bacitracin (Polyfax, Wellcome). For the trial, in addition to this routine, about half the patients were treated for 1 month with nystatin ointment mixed in equal parts with the Polyfax ointment; nystatin was then withdrawn for the second month. In the remainder of the patients the treatment was reversed, nystatin being used in the second month, but not in the first. After each period of a month the ulcers were treated for 1 week with Polyfax only. Scrapings from the surrounding skin and swabs from the ulcers were taken and examined by the laboratory at the end of each fifth week.

Reduction or loss of pain, and decrease in the area or depth of the ulcer were used in assessment of clinical improvement. The observer was unaware which ulcers were being treated with nystatin.

It will be seen that the yeast was eradicated in all cases when treated with nystatin, but the clinical response was not closely related to the disappearance of the yeast.

TABLE 1. Clinical and mycological results after each month of treatment

Treatment	No. of patients	Clinical improvement	Yeast eradicated
No nystatin in first month	7	5	1
Nystatin in second month		7	7
Nystatin in first month	10	10	10
No nystatin in second month		5	6

In four of the ten cases in whom the yeast was eradicated after one month's nystatin treatment, it recurred less than four weeks later. In one case the yeast disappeared in the first month, when no nystatin had been applied.

Trial 2

Sixteen of the patients in Trial 1 whose ulcers did not heal up during the 2 months treatment had regular swabs and scrapings taken at intervals of 6–8 weeks, and their clinical assessment was recordee. These patients were followed up either until the ulcers healed, or for a period of 1 year.

Treatment with nystatin added to the Polyfax ointment was continued in all cases for as long as the scrapings showed fungus to be present. The details of this second trial are not presented here, but it was clear that whether the yeast was quickly removed, whether it was removed and recurred later, or whether it persisted for a long time, had no obvious clinical effect on the rate of healing.

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Case Reports

Unusual ulceration in diabetes mellitus

JULIAN VERBOV

Broadgreen Hospital, Thomas Drive, Liverpool L14 3LB

Accepted for publication 7 June 1974

SUMMARY

A 35-year-old woman developed ischaemic changes over the soles of her feet within hours of admission to hospital moribund in hyperglycaemic coma. Skin and subcutaneous tissue separated but slow primary healing with scar tissue has taken place. An explanation for the plantar gangrene is offered.

CASE REPORT

Mrs M.R., aged 35 years, and not known to be diabetic, was admitted to the intensive care unit deeply unconscious and moribund in hyperglycaemic coma on 8 August 1973; her blood sugar was 1206 mg/100 ml. Her history, obtained from her later, was that 3 weeks previously she had developed blurred vision and this was followed by pruritus vulvae, thirst, polyuria and vomiting; unconscious only for the 24 h prior to admission she had managed to remain on her feet at home for much of the 3 weeks despite the vomiting, with periods of bed rest and sitting.

Four hours after admission early gangrenous change was visible over the toes, soles including the points of the heels but excluding the insteps (Fig. 1), and over a small area of the left buttock and of the left calf. She recovered with routine treatment for diabetic keto-acidotic coma; skin and some subcutaneous tissue in the areas mentioned gradually separated (Fig. 2). Although slow, healing with scar tissue has been progressive and uneventful apart from slight flexion contracture of the left big toe

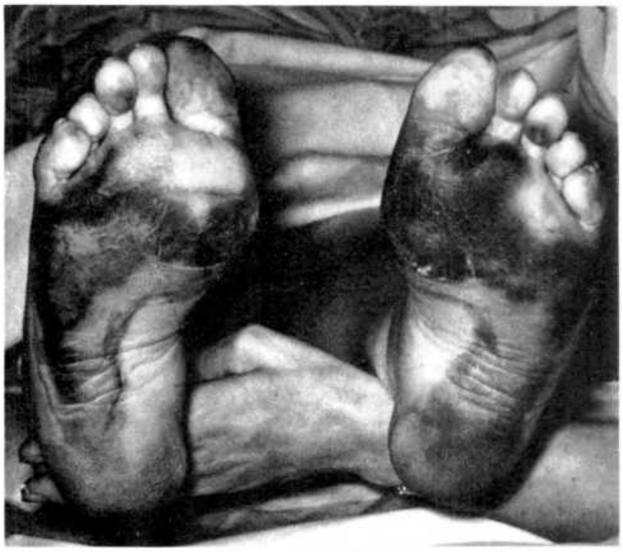
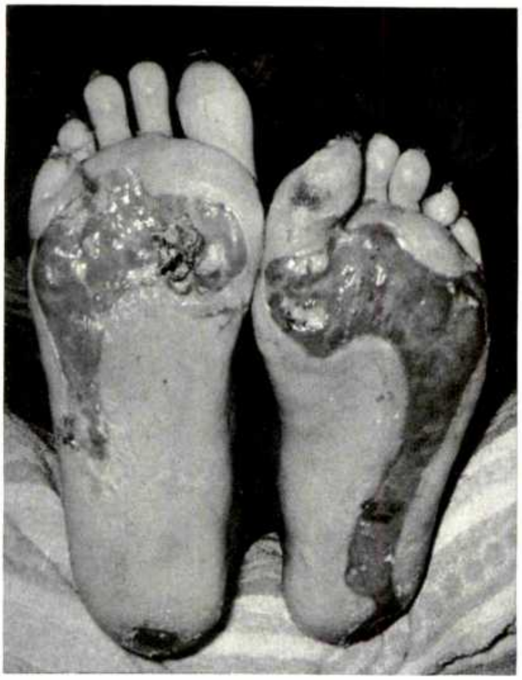


FIGURE 1. Ischaemic skin on soles 6 days after admission.



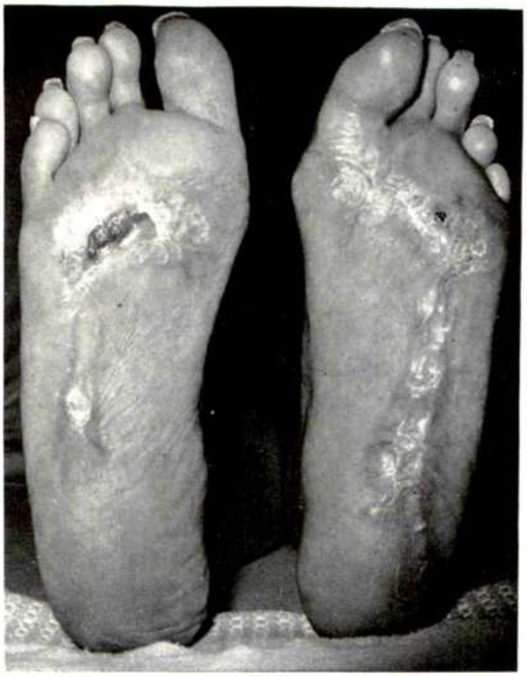


FIGURE 2. Ulcerated skin 3 months after admission.
FIGURE 3. Healing skin 8 months after admission.

(Fig. 3). She also has some impairment of light touch and pin-prick sensation over the soles (except the insteps) and lateral aspect of the left lower leg but peripheral arterial pulses are all present and normal; anterior and posterior tibial pulses were not palpable when she was admitted moribund. At present, her maintenance dosage of insulin is 36 u lente insulin daily. Treatment of the skin consisted initially of wet dressings of 2·5% aluminium acetate (aqueous solution) and finally 1% chlortetracycline ointment.

DISCUSSION

The bizarre ulceration of the soles in this new young diabetic is considered to be due to acute ischaemia as a consequence of hypotensive circulatory collapse; pressure on the left buttock, calf and points of heels when unconscious explains the gangrene in these areas. Pressure on the soles, whilst ambulant yet becoming progressively more hyperglycaemic, could well have predisposed to the plantar gangrene and necrosis that occurred with unconsciousness. The diabetic patient described by Riven (1935) also seems to have had this rare gangrenous skin change, but the bullous eruption occurring in diabetics described most recently by Allen & Hadden (1970) appears to be a different entity.

ACKNOWLEDGMENT

I should like to thank Dr B.A.Walker under whose care the patient was admitted.

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Acrodermatitis enteropathica treated by zinc

BENJAMIN PORTNOY AND MOHAMED MOLOKHIA

Skin Hospital, Manchester

Accepted for publication 20 May 1974

SUMMARY

A case of acrodermatitis enteropathica has, after 14 years essential treatment with Diodoquin, been able to abandon this therapy on exhibition of zinc.

Barnes & Moynahan (1973) reported a case of acrodermatitis enteropathica (AE) in a baby girl with multiple dietary intolerance treated by diiodohydroxyquinoline (Diodoquin), in whom the administration of zinc sulphate (3 mg daily) was promptly followed by complete disappearance of her skin lesions. The synthetic diet which had to be given was found to be grossly deficient in zinc and this was clearly reflected in the subnormal plasma zinc levels (Moynahan & Barnes, 1973). When zinc supplements were inadvertently omitted, the condition relapsed, only to clear after re-introduction of zinc.

CASE REPORT

We wish to report the results of zinc therapy in a 14-year-old girl with this condition.

When first seen aged 6 months, she had a polymorphic eruption of blisters and crusted erythematous lesions affecting her buttocks, face and neck. At first diagnosed as impetigo (other possibilities being dermatitis herpetiformis and epidermolysis bullosa), her morose condition and complete baldness raised the question of AE in spite of the absence of gastrointestinal abnormalities. She responded rapidly to Diodoquin (commencing with 400 mg daily) and was in fact the first case of AE without diarrhoea to be reported (Portnoy & Marsden, 1961).

One of us (B.P.) has seen this child at approximately 6 week intervals during the whole of her life. Her requirements of Diodoquin slowly increased until she was receiving 1·2 g daily. Numerous attempts at reducing this dose were invariably followed by hair loss (requiring a wig), exacerbation of skin lesions and a noticeable fall in her form position at school. Plasma zinc levels were estimated and were found to be well below the normal range—indeed the lowest encountered in our laboratory (Table 1). Tissue zinc levels were also estimated and were found to be nearly one and a half times the average normal range, the tissue being taken from unaffected skin on the back.

It was decided to give this metal as zinc sulphate (300 mg daily). Within a week, she began to show marked improvement and, within 3 weeks, all the skin lesions had cleared in a most dramatic manner for the first time in her life. A month later, it was decided to reduce the Diodoquin dose to 600 mg daily and, 1 month later this was withdrawn altogether.

Now, 5 months after commencing zinc therapy, her skin remains completely clear, and her nails,

TABLE 1. Zinc levels in skin and serum

Sample	Zinc concentration (μg/g dry weight)	Normal range and mean (Molokhia & Portnoy, 1973)
Epidermis	89·5	42·5–87·0 (56·6)
Dermis	19·8	7·7–18·8 (12·5)
Serum	5·35	7 54–13·80 (10·40)

which had always been abnormal, are growing satisfactorily. Her hair, which had been sparse, depigmented and of poor quality, now shows new growth which is strong and dark. In fact there is a clear line of demarcation between new and old hair and the eyebrows too are darkening and growing strongly. In those 5 months, the patient has gained 3·2 kg in weight and 4·5 cm in height and, according to her mother, is now enjoying a healthier appetite.

We believe that this clear-cut clinical response to zinc therapy, which has been maintained long after withdrawing Diodoquin, reflects an abnormality in zinc metabolism in this disease, a belief further strengthened by the finding of exceptionally low serum zinc levels. It is perhaps significant that some of the features found in AE are reminiscent of zinc deficiency states which have been described in man and animals (*Lancet*, 1968). The cause of this abnormality, however, is not clear. Unlike Moynahan and Barnes' case, there was nothing to suggest inadequate zinc supplies in the diet of our patient.

There is of course a possibility that treatment with Diodoquin might have been a contributing factor. Like other quinoline derivatives, this substance is a potent metal binding agent and it could be that the large amounts of this drug, given orally, were causing chelation of dietary zinc. If indeed it can be regularly demonstrated that zinc plays an important part in the treatment of AE, it would seem paradoxical that Diodoquin could be beneficial. Until now there has been no satisfactory explanation of the mode of action of Diodoquin and, although it was originally thought to be acting locally, it is now known to be absorbed from the intestine and indeed to give rise to retinal complications (Garcia Perez *et al.*, 1974).

Under these circumstances, it is conceivable that a certain amount of dietary zinc, hitherto unavailable, is transported by Diodoquin in a bound form through the intestinal barrier where it may serve a limited role in tissues. Therefore, we suggest that this drug is acting purely as a zinc carrier. To Moynahan, Johnson & McMinn (1963) must go the credit for showing an enzyme defect in the intestinal mucosa in AE. This defect could be related to an inborn error in the absorption of zinc from food, in a manner similar to that found in relation to copper in Menke's Kinky Hair Syndrome (Danks, Campbell & Stevens, 1972). It would be of interest in any new case of AE to estimate serum zinc levels and, if found low, to give zinc instead of Diodoquin.

The success of zinc therapy in this disease would be a welcome development as it eliminates the risks of the retinal complications of Diodoquin and also opens the way for a better understanding of the role of zinc in the body. It is our intention to estimate zinc in the new and 'old' hair and to repeat zinc determination in serum and tissues.

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Comment

DYNAMICS OF MULTINUCLEATE GIANT CELL FORMATION, *IN VIVO* AND *IN VITRO*

Multinucleate giant cells (MGC) have long been recognized to be a characteristic component of many granulomatous infiltrates. Ever since Langhans (1868) discussed in some detail the role of MGC in tuberculosis and foreign-body reactions there has been continued speculation on the mode of their formation. The purpose of this communication is to outline the present evidence concerning the dynamics of MGC formation in granulomatous infiltrates as well as *in vitro* systems.

(*I*) *Foreign-body granulomas*

Autoradiographic studies have indicated that MGC are derived from circulating mononuclear cells (Gillman & Wright, 1966). Carter & Roberts (1971) also believed that MGC are formed by fusion of non-proliferating macrophages. Recently Papadimitriou, Sforsina & Papaelias (1973) have studied the kinetics of MGC formation in foreign-body reactions still further, as well as the effects of various agents on their formation. Giant cell formation was induced by implanting strips of Melenex™ plastic into the subcutaneous tissue of mice. The implants were removed at variable intervals and the proportion of MGC as well as the number of nuclei they contained were counted and statistically assessed. A fusion index was calculated as the percentage, in a given sample, of the fraction of nuclei within MGC to the total numbers of nuclei in the cells counted. Papadimitriou *et al.* (1973) found that fusion occurred rapidly amongst the population of attached mononuclear cells and that the fusion index rose from 5% on the third day to 45% on the seventh day. Thereafter, this high level of fusion remained unaltered until the end of the period of implantation (8 weeks). Total body irradiation to the mice reduced the circulating monocytes to a low level, thus enabling a better estimate to be made of the fusion potential of those mononuclears already attached to the Melenex. In this situation the highest fusion indices were found. Treatment with actinomycin D reduced the fusion index and the mean number of nuclei within MGC, suggesting that the fusion is partially dependent on DNA transcription. Perhaps surprisingly, puromycin (a compound which interferes with protein production) caused an increase in both fusion index and the proportion of MGC present. Corticosteroids did not greatly influence the fusion process. When attached monocytes were stimulated to phagocytose carrageenin their fusion potential was inhibited. Papadimitriou and his colleagues' experiments (1973) indicated that the life span of MGC was short (in the range of a few days) and that the more nuclei the cell possessed the older it was likely to be. Ryan & Spector (1970) found that DNA synthesis could occur in the nuclei of MGC so it is just possible that mitoses without cellular division could account for the formation of some MGC.

(*II*) *Organized epithelioid cell granulomas*

Pathologically, epithelioid cell tubercles consist of three main cell types, undifferentiated mononuclear cells (also called 'activated lymphocytes' by Jones-Williams *et al.*, 1971; Jones-Williams, Fry & James, 1972), epithelioid cells and MGC. Autoradiographic studies have shown that tubercles are dynamic organs in which mononuclear cells constantly divide and differentiate into epithelioid cells (Epstein & Krasnobrod, 1968; Epstein, 1971). Jones-Williams *et al.* (1970, 1971) have proposed that two types of epithelioid cells exist in tuberculoid granulomas. They identified an 'A' or secretory type of epithelioid cell which contained abundant rough-surfaced endoplasmic reticulum and distinguished this type from a 'B' or vesicular epithelioid cell which was characterized by the presence of numerous vesicles within its cytoplasm. Until recently the histogenesis of MGC within tuberculoid granulomas had not been thoroughly investigated, although it had been generally assumed that they were formed by fusion of epithelioid cells (Fallis, 1964; Wanstrup & Christensen, 1966; Azar & Lunardelli, 1969; Jones-Williams *et al.*, 1970, 1971, 1972). Black & Epstein (1974) have recently studied the formation and role of MGC in metal-induced granulomas. Organized epithelioid cell granulomas were produced experimentally by injecting intradermally dilute suspensions of zirconium and beryllium salts into individuals who had previously been sensitized to these metals. Biopsies were obtained at intervals up to 13 months later and the specimens were fixed and prepared for light and electronmicroscopy. Tritiated thymidine was injected into some of the granulomas; the biopsy specimens were taken 40 min to 2 or more weeks later, and the tissue processed for light microscopic autoradiography. MGC occurred commonly, both within organized tubercles and in relation to areas of necrosis. They were observed as soon as the granulomas became organized, particularly within oedematous disrupted tubercles and adjacent to necrotic areas, supporting the previous suggestion that MGC develop in a 'toxic environment' (Davis, 1963; Jones-Williams *et al.*, 1972). Ultrastructurally, these MGC had markedly different cytoplasmic features from typical secretory epithelioid cells which enabled them to be readily recognized at scanning magnifications. The characteristic hallmark of these MGC was the presence of myriads of small mitochondria adjacent to nuclei and of numerous membrane-lined vesicles in the centre of the cytoplasm. MGC occurred mainly in oedematous tubercles in which the epithelioid cells contained similar cytoplasmic components to the MGC (vesicular epithelioid cells). Although direct evidence of cell fusion was not seen, apparent fusion of plasma membranes seemed to occur between epithelioid cells and MGC. No nuclear labels were found in any MGC 40 min after injection of tritiated thymidine, indicating that further nuclear division does not occur within MGC. The ultrastructural evidence suggests that in organized epithelioid cell granulomas the majority of MGC are formed from fusion of vesicular rather than secretory epithelioid cells.

(*III*) In vitro *systems*

The appearance of MGC in monocyte cultures has been known for many years and has been considered to be a phenomenon found only in ageing cultures (Sutton, 1967; Hanifin & Cline, 1969). Recently, Black, Fukuyama & Epstein (1974) have shown that MGC in fact appear in human blood monocyte cultures at a very early stage. In a small proportion of normal adults the numbers of MGC present were consistently higher than in the others. Supernatants from early monocyte cultures of those subjects having a high incidence of MGC induced the formation of MGC in other cultures. The effect of this factor in inducing MGC formation occurred relatively quickly (<2 h). These preliminary experiments have clearly demonstrated the presence of a biochemical mediator which is capable of inducing the formation of MGC in other monocyte cultures. The biochemical nature of this mediator has yet to be elucidated, nor is it clear whether it is secreted by the monocytes or contaminating lymphocytes in the particular *in vitro* system used. The mediator is unlike some other factors secreted by monocytes (e.g. colony-stimulating factor) which exert their effect by inducing synthesis of DNA (Chervenick & Lo Buglio, 1972; Golde, Finley & Cline, 1972). Furthermore, Comoglio, Ottino & Cantino (1971) have also shown that high molecular weight compounds are necessary in the culture medium for MGC to develop *in vitro*.

Until now most studies have been concerned with the kinetics of MGC formation and little is known about their role in granulomatous infiltrates. Black & Epstein (1974) have postulated that in tuberculoid granulomas the appearance of MGC and vesicular epithelioid cells may be an attempt by the host to terminate the granulomatous process, preceding the healing by fibrosis. Papadimitriou *et al.* (1973) have shown that in foreign-body granulomas MGC are also short-lived, so it is possible that similar host mechanisms may be operating. However, much more work needs to be done.

both *in vivo* and *in vitro*, before the role of MGC in granulomatous infiltrates is finally understood.

MARTIN M.BLACK

Department of Histopathology,
Institute of Dermatology,
London W.C.2

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ADDENDUM

Since this manuscript was submitted Mariano & Spector (1974) using light and electromicroscopy, autoradiography, chromosome analysis and cell culture techniques, have recently published their results in a study of the formation of inflammatory giant cells (macrophage polykaryons), also on glass or plastic coverslips inserted subcutaneously into mice. Mariano & Spector postulate that multinucleate giant cells form at sites of inflammation by fusion of newly arrived macrophages with macrophages already *in situ*, many of which are dividing and exhibit chromosomal abnormalities. Like Papadimitriou, Sforsina & Papaelias (1973) inflammatory giant cells were found to have a short life span so Mariano & Spector suggest that the formation of giant cells may be an efficient disposal mechanism for unwanted macrophages.

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Society Proceedings

North of England Dermatological Society

3 April 1973, Leeds

President Dr D.I.McCallum

Tropical Fish Tank Granuloma Dr K.Keczkes (Hull)

A man aged 26 years, fish worker.

History. In mid-January 1972, erythema and swelling developed on the dorsum of his right hand; during the subsequent 2 months, it spread in linear fashion to the middle of his right arm. Symptoms were practically absent.

Employed as a fish-worker in the fish docks, he also kept tropical fish as a hobby, at home; in the previous few months, some of his fish had died.

On examination. Red, raised, indurated, nodular lesions were present on the back of his right hand, and a linear chain of nodules extended from the hand, up the forearm, to reach the middle of the right arm (Fig. 1).

Investigations. The Heaf test was positive.

Skin biopsy showed a granuloma in which no fungal elements were demonstrable by special stains, and no acid-fast bacilli were visible after Ziehl–Neelsen staining.

Bacteriological studies produced a profuse growth on Lowenstein–Jenson medium, after 2 weeks' culture at 22°C; the colonies developed a yellow pigment when exposed to daylight. The organism was identified as *Mycobacterium marinum* by Dr J.Marks at the Public Health Laboratories.

Treatment comprised sulphamethoxazole-trimethoprim tablets, two twice-daily, and the application to the right arm of a special, polythene covered, insulated, electrically heated armlet which was worn for 12–16 h each day. The lesions gradually resolved and had disappeared by the time treatment was stopped after 6 months.

Comment. As this condition can be very resistant to therapy, it was felt that the electrically heated armlet was an advance in therapy.

12 June 1973, Middlesbrough

President Dr J.K.Morgan

Iododerma Dr Inman

A man aged 45 years.

History. First seen in September 1971 with sterile pyodermatous lesions on his face, the patient subsequently developed a few more on his trunk (Figs. 2 and 3).

A clinical diagnosis of iododerma was supported by a compatible histology, and by the fact that his wife had been giving him iodized salt because her mother had had a goitre.

The lesions responded quite well to sodium chloride capsules which, however, the patient has been loathe to take.

Course. Subsequent events, including the way in which the diagnosis was proved, were remarkable. In addition to the lesions of his skin, he not only developed nodules in his eyelids which damaged his eyes, but also became partially deaf and suffered from headaches. A neurologist was consulted, who opined that the symptoms were functional as a result of damage to the eyes of a person with a sensitive temperament. This view the patient's wife disputed, and she persuaded the neurologist to do a cerebral

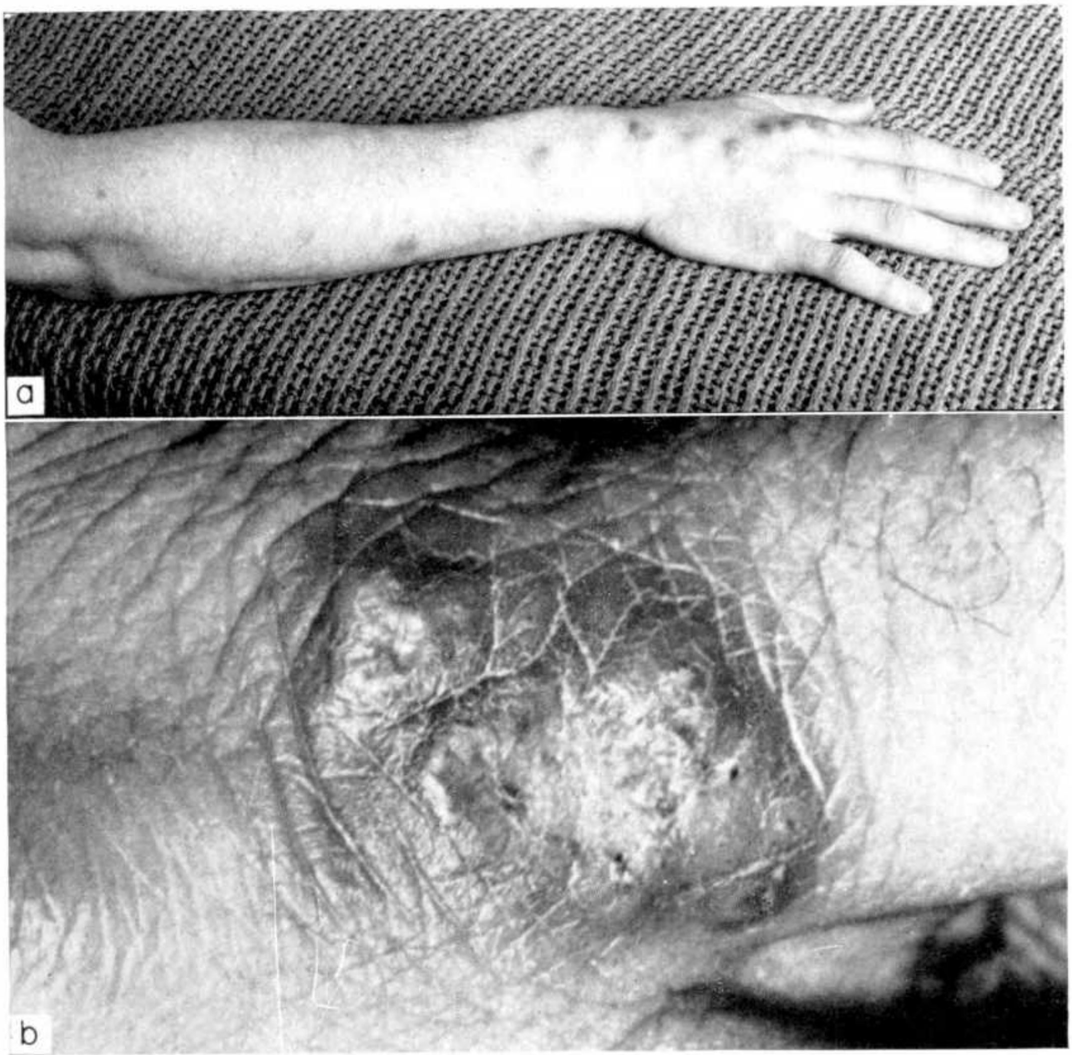

FIGURE 1a, b

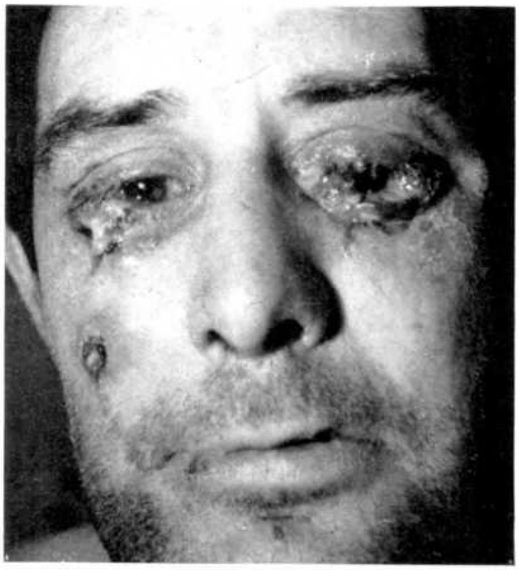

FIGURE 2

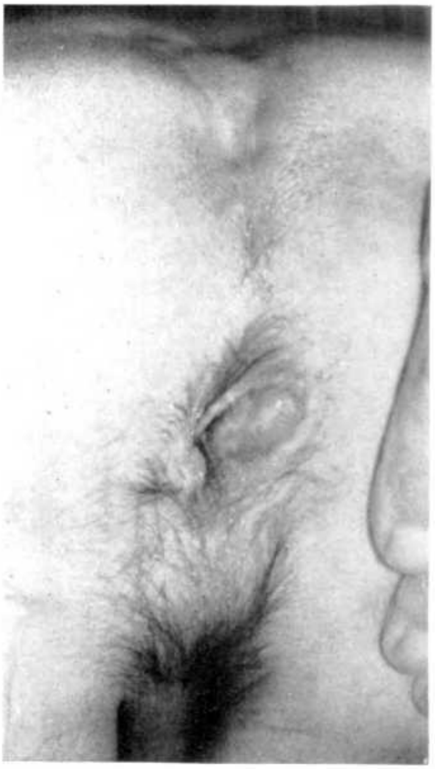

FIGURE 3

scan to exclude a cerebral tumour. Radioactive iodine was used in the test—no sign of a cerebral tumour was found, but all the iododerma lesions promptly recurred.

Destruction of the sclera has occurred to a degree sufficient to make rupture of the globes a distinct possibility; in fact, the patient is practically blind.

9 October 1973, Liverpool

President: DR J.K.MORGAN

Pseudohypoparathyroidism and Pseudo-pseudohypoparathyroidism DR NETTA HAY (Shown by DR DAVE)

A mother with two of her daughters aged 1 and 11 years.

Family history. Three members of this family are normal: the father; the second daughter, aged 10 years; and the third daughter, aged 2 years 10 months. A fourth member, the elder son, aged 7 years, has bilateral hydronephrosis but is otherwise normal.

Two children, the younger son who died at the age of 3 years in July 1973, and the youngest daughter, aged one year, have been affected by pseudo-pseudohypoparathyroidism; and it is possible that the mother, also, has this complaint.

The other member of the family, the eldest daughter, aged 11 years, has pseudohypoparathyroidism.

History. (a) The younger son's abnormalities drew attention to disease in the family. At the age of

3 years, he was noted to have broad hands, and purplish erythematous areas and subcutaneous plaques on his trunk. He was mentally retarded and had a patent ductus arteriosus.

Skin biopsy showed 'soft tissue calcification with a trace of mucin deposition'. No abnormality of calcium metabolism was found.

He was diagnosed as having pseudo-pseudohypoparathyroidism.

He died, during a syncopal episode, in July 1973.

(b) The youngest daughter, aged 1 year, has had, from the age of 5 months, a purplish erythematous plaque on her left thigh, and others on her trunk. Her face is round, and her length is on the third percentile.

Her serum calcium, phosphorus and alkaline phosphatase are normal.

X-rays of her skull and hands show no abnormality.

In a skin biopsy, dermal calcification was seen.

She was diagnosed as having pseudo-pseudohypoparathyroidism.

(c) The eldest daughter, aged 11 years, has no visible abnormality of her skin. Her stature is short and she has a round face, hypoplastic teeth, and short metacarpals as a result of which dimpling of the dorsa of her hands occurs on flexion of her fingers. The spaces between her enlarged first toes and her second toes are wide. She is mentally retarded.

Biochemical analysis of her serum has given the following levels: calcium 7·5 mg/100 ml; magnesium 2·1 mg/100 ml; phosphate 7·2 mg/100 ml; alkaline phosphatase 26 KA units.

X-rays of her hands and feet showed that some of the metatarsals and metacarpals are short, that there are some bone cysts, and that a little soft tissue calcification is present adjacent to the distal joints.

A diagnosis of pseudohypoparathyroidism was made.

(d) The mother has a short stature.

X-rays of her hands showed the metacarpals of her ring and little fingers to be short, and the presence of periarticular calcification near the metacarpo-phalangeal joints of her index and middle fingers.

Serum calcium and phosphate levels were found to be normal.

It is thought that she may have pseudo-pseudohypoparathyroidism.

Comment. (1) In pseudohypoparathyroidism the tissues are resistant to parathormone and the biochemistry is *abnormal.*

(2) In pseudo-pseudohypoparathyroidism the biochemistry is *normal,* although there are physical stigmata of pseudohypoparathyroidism.

(3) It has been noted previously that both pseudohypoparathyroidism and pseudo-pseudohypoparathyroidism may occur in the same family.

Skin Microbiology Club

The summer meeting of the Club was held at the Bristol Royal Infirmary on 25 July under the guidance of Professor W.A.Gillespie and Dr V.G.Alder. Papers presented were: Absorption of hexachlorophene from newborn infants' skin by Professor Gillespie; Unusual virulence of a strain of multi-resistant *Staph. aureus,* by Dr K.Joy Harrison; Neutral red in the treatment of herpes genitalis, by Drs Roome, Tinkler, Hilton, Montefiore and Waller; Therapeutic implications of plasmid carriage by *Staph. aureus,* by Dr R.Lacey. There was a full and lively discussion.

The next meeting of the Club will be on 9 January 1975 when the Club holds a joint meeting with the Pathological Society of Great Britain at the Middlesex Hospital, London. Anyone interested in attending this meeting should contact Dr W.C.Noble, St John's Hospital for Diseases of the Skin, London E9 6BX.

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Correspondence

EXPERIMENTAL *TRICHOPHYTON* INFECTIONS IN HUMANS AND SOME OBSERVATIONS ON THE BIOLOGY OF *T. RUBRUM* INFECTIONS

SIR, This refers to the above subject on which I have been quoted by Singh (1973) and by Barlow & English (1973).

Since only a few of my studies have been part-quoted, I crave the indulgence of your columns to recapitulate on this subject and correct some of the statements made by these authors. I carried out experimental and clinical observations to understand better the biology of *Trichophyton rubrum* infections in tropical conditions. The epidemiological evidence of its highly contagious nature, including husband–wife infections and the peculiarly high susceptibility of adolescents and adults compared to children, were demonstrated (Desai & Bhat, 1961; Desai, 1966). In publications on experimental *T. rubrum* infections (Desai & Bhat, 1960; Desai *et al.*, 1963), it was shown that traumatic abrasions were not required for an experimental take. Simple protection of the inoculated area with plain gauze moistened with saline or water for a few days and covered with adhesive tape gave consistently satisfactory results. Mycelial inocula gave better takes than spores. The importance of the mycelial phase in extension of the disease was also illustrated. In Table 1 I now summarize other observations on the nature of experimental disease in more than thirty healthy volunteers of various age groups compared with subjects with chronic infections, both at the primary and the subsequent inoculations carried out after 6 months and after 2 years. The results are correlated with their trichophytin status.

I also received twelve publications on experimental dermatomycosis from which the nature of the immunological response was conjectured as of 'natural type' (Desai, 1963b).

TABLE 1. Natural course of experimental ringworm infection in humans (*T. rubrum*)

Experiment	Children	Adolescents	Adults
First inoculation			
Incubation period	8–10 days	6 days	2–3 days
Duration of disease	65–70 days	35 days	14–35 days No self-termination in abnormal subjects
Second inoculation after 6 months and 2 years			
Incubation period			2–3 days
Duration of disease			14–35 days No self-termination in abnormal subjects
Trichophytin status and duration of disease			
Normal reactivity			14–35 days
Hypersensitivity			60–75 days
Negative anergy			No self-termination in abnormal subjects

Investigating some factors which may be responsible for recurrent and chronic ringworm infections of 2–20 years' duration in some of my subjects, I found that many patients had dysproteinaemia with hypergammaglobulinaemia suggesting that circulating antibody defences were probably not involved. Some of the latter subjects had negative immediate and 48-hour type of trichophytin reactivity (Desai *et al.*, 1962) while others had only immediate positive reactivity (unpublished). Histologically, some subjects with chronic ringworm showed an absence of the leukocytic defence in the subcorneal keratin layers which was observed in subjects with successful defence (Desai, 1972)—(if confirmed, this may now be explainable on the basis of absent cell-mediated immunity on which then we had no knowledge). Serum fungistatis was investigated in the subjects with adequate and inadequate defence and gave variable results and did not explain the chronicity (Desai & Harvey, 1967). I observed mycelial invasion of vellus hair follicles in the affected glabrous skin, giving rise to what may be termed 'follicular trichophytosis without hair invasion' (unpublished). In some of the subjects with extensive glabrous skin ringworm this is possibly one of the factors responsible for chronicity and recurrence.

From the above data, and comparing the immunity in skin ringworm with that as understood in tuberculosis, which was the model for understanding acquired immunity, I postulated that the resistance to ringworm infection of the skin is likely to be of the natural type and not of the acquired variety, as the primary infection does not modify the behaviour of subsequent infection as in Koch's phenomenon (Desai, 1963a).

SHARAT C.DESAI

Medicare Research Clinics,
105/106, Maker Bhavan No. 3,
21, New Marine Lines,
Bombay,
India

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5-FLUOROCYTOSINE TREATED CHROMOMYCOSIS

SIR, I have read with interest the article on successful treatment of chromomycosis with 5-fluorocytosine (5-FC) which appeared in the April issue of your journal (Morison, Connor & Clayton, 1974). There are, however, some points which are open to comment.

Firstly, the largest reported series treated with oral 5-FC is that of Lopes *et al.* (1971) who, out of twelve patients treated, obtained a cure in five (41·6%). These investigators noted that resistance developed to 5-FC and increasing the dose to double the normal i.e. 200 mgm/kg/day produced no further clinical improvement. In fact, the lesions continued to deteriorate. The follow-up period in this series was 3–10 months. Although Magalhaes & Meyer (1970) reported a 100% cure rate in ten patients, these patients were not followed up.

During the last 4 years I have treated twelve patients solely with oral 5-FC (unpublished data), obtaining a cure in only 3 (25%) with a follow-up period varying from 6 months to 2 years. Resistance to 5-FC was noted both before exposure to the drug and also during treatment. Again, confirming Lopes' findings, increasing the dose of 5-FC to double the normal produced no further clinical improvement. The lesions continued to extend and spread.

My earlier experience (Bayles, 1971) in the treatment of this chronic disease using thiabendazole resulted in a cure rate of 36·4% in twenty-two patients. There were no recurrences during a follow-up period of 4 years except in one patient who had a late recurrence 11 months after discontinuation of treatment. The drug was well tolerated in the majority of cases but a hypersensitivity reaction developed in six of the twenty-two patients. All abnormal biochemical findings reverted to normal following discontinuation of the drug.

Although the long term outlook for patients with chromomycosis has improved considerably with the introduction of these two drugs, it would appear from our discussion following the Round Table on the Therapy of Chromomycosis at the 9th International Congress on Tropical Medicine and Malaria in Athens, October, 1973 that no single chemotherapeutic agent is likely to provide the answer to this particularly resistant disease.

M.A.H.BAYLES

King Edward VIII Hospital,
Congella,
Durban,
S. Africa

A REPLY

SIR, Our intention in the original article (April, 1974) was to report the successful use of 5-fluorocytosine in a single case and we did not wish to imply that we were conducting an adequate clinical trial of the drug. This letter emphasizes the necessity of always testing the sensitivity of the organism to 5-fluorocytosine because resistance has been reported and an infection cannot be expected to respond if the organism is resistant. In addition, perhaps better results would be obtained if serum levels of the drug were monitored and the dose adjusted accordingly.

WARWICK L.MORISON

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ACRODERMATITIS ENTEROPATHICA

SIR, I am prompted by the rarity of acrodermatitis enteropathica to add a comment on the interesting case report from Dr Portnoy and Dr Molokhia (see p. 701 of this issue) which they have kindly allowed me to see.

I wish to report the case of a male infant when aged 6 months who was seen by me. The child was bottle fed, well nourished, but with somewhat loose stools and the dermatological complaint was a persistent erythema and pustulation which extended around the anal orifice. This had appeared within a few weeks of birth and had not varied in severity. It had failed to respond to anticandida topical applications prescribed by the family doctor.

Examination revealed a well-nourished boy with a micro-pustular eruption confined to an area which extended 2–3 cm around the anus. No other lesion could be found in the mouth or elsewhere on the skin. Cultures from the affected area were negative for candida but despite this oral nystatin and Canesten ointment were tried for some 6 weeks. The lesion remained unchanged, and in the belief that this might be a very mild example of acrodermatitis enteropathica and in the light of Dr Moynahan's work, a plasma zinc estimation was carried out. This revealed an extraordinarily low figure of 17 μg/100 ml, the normal range being 60–190.

In view of this the infant was given 50 mg of zinc sulphate daily and within a week the perianal skin lesion had cleared and the mild diarrhoea ceased.

Three weeks after commencing the zinc, a further estimation of plasma zinc was made and this gave a figure of 84 μg/100 ml.

I have no proof that this was an example of acrodermatitis enteropathica but the perianal pustular eruption was an unusual one and the plasma zinc level was extraordinarily low. The fact that the patient recovered with zinc alone when no diodoquin was given is an interesting point.

Rupert Hallam Department of Dermatology,
Sheffield

IAN SNEDDON

Book Review

The diagnosis and treatment of fungal infections. HARRY M.ROBINSON JR. (Comp. and Ed.) Springfield, Illinois: Charles C.Thomas. Pp. 560, Numerous illustrations, some in colour. Price £12.59.

This volume, Professor Robinson explains, 'is a compilation of articles representing current thought on various topics' mainly from those who have contributed to symposia at recent Annual Meetings of the American Academy for Dermatology. He stresses that he and his thirty-nine associates have not attempted to produce a comprehensive textbook. As compiler, Professor Robinson must be congratulated on the sheer number of presentations and the choice of topics. The best of them are warmly recommended. Drake & Maibach's opening chapter on candidiasis is an extremely penetrating review which could hardly be bettered. There is a useful, well-referenced paper on tolnaftate by Philip Charney; and Shah, Epstein & Riegelman have covered griseofulvin equally well. Levan's 'Coccidioidomycosis' is an up to date introduction worthy of note. For those seeking 'something completely different' there is Frances Keddie's 'Tinea Versicolor 1846–1900' which provides a pleasantly illuminating glimpse of the evolution of ideas about host/parasite relationships.

In the clinical papers generally there is a rather disappointing lack of personal authority and wisdom which such a book might be expected to provide, but two chapters are exempt from this criticism.

Chenovsky's 'Differential Diagnosis of Pigmented Dots of the Scalp' pushes the art of differential diagnosis to the extreme limits and merits attention, as does the chapter in which Tindall & Elson describe their experience of tinea faciale. They draw attention to the apparently common exacerbation of this condition by sunlight, an event which often leads to the misdiagnosis of discoid lupus erythematosus—an important practical observation. There are several other valuable contributions but it must be said that many are no more than mediocre. One or two could have been written by anyone with access to the standard texts, and are frankly dull. Others are accounts of experimental work either too slight or too preliminary for inclusion within hard covers.

There are contradictory statements of fact, see especially Chapters 22 and 24, occasional omissions such as a table from Chapter 1, and a very variable, sometimes unacceptably low, standard of referencing. Professor Robinson, in his role as editor, should certainly have directed his team more firmly. Had he done so we might not have been treated to the appearance in Chapter 24 of the 'conc. of gris.' For a brief and faintly pleasurable moment I thought he must be the sovereign and bejewelled ruler of some minute exotic island kingdom. However reflection brought enlightenment, and amusement gave way to irritation as this ugly abbreviation appeared again and again. The value of the book would have been greater had the index reached a reasonable standard of competence but its five and a half pages do less than justice to the 550 pages of text. This is a volume which those interested in the clinical and therapeutic aspects of the mycology of the skin will want to consult occasionally. Postgraduate students can confidently be recommended to read selected chapters but the book as a whole is less stimulating and rewarding than the list of contents would suggest.

S.O.B.Roberts

British Journal of Dermatology (1974) **91,** 719.

Review

Parenteral nutrition

Parenteral nutrition, a term which describes the provision of nutrients including fat, protein and carbohydrates to the body by the intravenous route, is known also as intravenous feeding or hyperalimentation (Dudrick *et al.*, 1969). It is an aspect of the care of the severely ill which has achieved some importance during the past 20 years. Before that time the nutritional management of the sick was catered for either by the resilience of the human frame to withstand prolonged starvation or by the culinary skills and powers of persuasion of the dietitian or ward sister. The increase in the use of this technique has gained impetus from the relatively recent development of life support systems in intensive care where there is often a need to nourish a patient unable to take food by mouth for lengthy intervals because of sedation and endotracheal intubation. In the general clinical field this method of feeding is being used more frequently as the importance of starvation, partial or total, in potentiating metabolic abnormalities has become more widely appreciated (Moore, 1958).

Up to the present time the major advances have been made by the pharmaceutical industry in producing stable solutions of fat, protein (as amino acids) and carbohydrates, which are non toxic and readily assimilable by the body. In practical terms the clinical technique is simple, involving intravenous cannulation of either a peripheral or a central vein. Factors which have limited the clinical development of parenteral nutrition have been lack of familiarity with the routine nutritional needs of the patient and a lack of knowledge of the subtle changes which occur in the manner whereby the body deals with common nutrients in the presence of acute disease. Advances to be anticipated during the next decade will be in this aspect of intermediary metabolism, to define how these subtle changes may be avoided or used to the patient's advantage.

Although widespread clinical use of parenteral feeding is relatively recent many attempts have been made during the past 100 years to nourish patients by the intravenous route. Carbohydrates have the longest record of use and this has been extensively reviewed by Thoren (1963). They have been given either as isomolar solutions, e.g. 5% glucose, at which level their caloric contribution is small, or as hyperosmolar solutions, from 10 to 50%, where the caloric contribution will range from 400 to 3000 kcal/l. Glucose is the ideal carbohydrate in most cases of short-term parenteral nutrition and is probably the most popular (Froesch & Keller, 1972). In situations of stress or in the presence of an increase in catabolism, such as severe burns, trauma or acute infection, the tolerance to glucose is reduced and insulin may have to be given to facilitate entry into cells (Allison, Hinton & Chamberlain, 1968). Because of this, fructose and sorbitol, a polyol which is metabolized to fructose, are also used as carbohydrate sources and are less dependent on insulin for efficient utilization (Froesch & Ginsberg, 1962; Bye, 1969; Lee *et al.*, 1972). In view of the reduction in the disappearance rate of glucose in stress and catabolism the current practice is to use more than one source of carbohydrate when large quantities are being administered and thus avoid overloading any one metabolic pathway (Bassler & Bickel, 1972).

Fat is provided as an emulsion of a vegetable oil, either soya bean as in Intralipid (Vitrum) or cotton seed as in Lipiphysan (Servier), where the triglycerides have been reduced to a particle size similar to that of chylomicrons. These particles remain stable in solution in the presence of an emulsifying agent and the final solution is isosmolar and non irritant (Schuberth, 1963). Fat provides approximately 9 kcal/g and therefore the caloric content is high, the commonly used 10% solution providing nearly 1000 kcal/l (Hallberg, Schuberth & Wretlind, 1966; Reid, 1967). To avoid overloading, which leads to depression of liver function and bone marrow, doses of fat in excess of 2g/Kg body weight/day should be avoided. In Europe, nutritional energy can be provided in parenteral nutrition regimes from a combination of carbohydrates and fat, almost half the total caloric requirements being in the form of carbohydrate (Froesch & Keller, 1972). However, it would appear that for short-term therapy the total caloric needs can be provided by carbohydrates alone. Should the duration of treatment extend into months there is risk of a deficiency of essential fatty acids developing (Collins *et al.*, 1971) and intermittent administration of fat is necessary (Jeejeebhoy *et al.*, 1973). Because patients who require parenteral nutrition are almost by definition physically inactive, the total daily caloric needs are related solely to the energy required for efficient utilization of the protein administered and little account need be taken of muscular effort. This means that approximately 200 kcal are needed for every gram of nitrogen given and the average daily requirement is between 2000 and 2500 kcal (Kinney, 1960).

Protein solutions must be presented as amino acids for efficient utilization and all solutions in common use contain the eight essential amino acids, with varying proportions of non-essential amino acids (Wretlind, 1973). The composition depends largely on the method of manufacture. Where amino acids have been btainedo from the hydrolysis of casein, e.g. Aminosol (Vitrum), almost all the amino acids are present with a small proportion of the smaller polypeptides (Wretlind, 1952). More recently, solutions of synthetically prepared 'l' amino acids have become available, such as Vamin (Vitrum) and Aminoplex (Geistlich). These have tended to replace the earlier synthetic solutions of amino acids, Trophysan (Servier), which contained many of the acids in the form of the 'l' and 'd' isomers. Utilization is more effective when all the amino acids are present in the naturally occurring 'l' form. These solutions are available in varying concentrations, commonly providing between 8 and 12 g nitrogen/l, a range which is sufficient to accommodate most needs. Two solutions, Aminoplex 5 and Aminosol–Fructose–Ethanol, have sufficient carbohydrate calories in the solution to achieve utilization of administered protein without additional energy. The total protein needs of a 70 Kg man are represented by an intake of 10–12 g nitrogen/day (Cuthbertson, 1972). It is probably better practice to express these requirements as 0·14–0·18 g nitrogen/Kg body weight/day which enables ready matching of the requirements over the large range of body weight encountered in clinical practice. Frequently in the presence of acute disease there is an increase in catabolism which may double or triple the protein requirements per Kg body weight but as this state is frequently associated with severe weight loss the total quantity needed daily rarely exceeds that for the normal individual (Hindmarsh & Clark, 1973; Clark, 1971).

The technique for infusing parenteral nutrition varies widely. A peripheral vein can be used for short term infusion but the risk of phlebitis is high, especially with hyperosmolar solutions and the current trend is to opt more and more for central venous cannulation via either one of the arm veins or the subclavian vein (Dudrick *et al.*, 1970). When large vein cannulation is used it is essential that the position of the catheter tip should be checked radiographically (Johnston & Clark, 1972) and that strict aseptic precautions are employed to reduce the risk of infection which may be as high as 10–20% (Bolasny, Martin & Conkle, 1971; Glover, O'Byrne & Jolly, 1971). Metabolic complications may occur during infusion and lactacidosis has been reported on more than one occasion, especially in neonates. This may be related to the rate of fructose administration or to the acid component of the protein solution (Bergstrom, Hultman & Roch-Horlund, 1968; Harries, 1972).

It is not possible to specify exactly conditions in which parenteral nutrition is indicated. Starvation can arise in almost any clinical situation which becomes complicated and it must remain a matter of judgement when to employ this method of feeding. Prerequisites should be that the patient is unable to meet his nutritional requirements and that no suitable alternative technique such as nasogastric intubation, gastrostomy or jejunostomy, can be used to maintain nutrition (Hindmarsh & Clark, 1973). Starvation, either partial or total, of only a few days duration leads to very few problems and does not call for parenteral nutrition but once present for more than 7 days serious consideration should be given to parenteral nutrition. Factors such as extreme catabolism, which increase the rate of utilization of body stores, will make the decision more immediate (Clark, 1973).

Obvious situations where parenteral nutrition is beneficial include the seriously ill requiring intensive care, patients with high intestinal fistulae associated with chronic inflammatory disease of the small or large intestine, and following major surgery for malignancy (Clark & Lauder, 1969; Wilmore *et al.*, 1969; Truelove & Jewell, 1974). Satisfactory nutrition has been maintained by parenteral feeding over periods of 2–3 months. Extensive burns create a serious nutritional problem because of limitation of the dietary intake by anorexia and the extensive protein losses by excess catabolism and exudation. Long term parenteral nutrition has been used successfully by Liljedahl (1972) to prevent serious weight loss and achieve satisfactory skin cover in patients with burns of 75–85% of the body surface area. A recent development has been the use of this method of feeding in the management of the uraemic patient, where urea nitrogen may be re-cycled for protein synthesis provided a supply of essential amino acids with histidine is given with a high carbohydrate intake (Josephson *et al.*, 1970). This achieves a reduction in blood urea levels and prevents serious deterioration in the nutritional status of the patient.

A more controversial use of parenteral nutrition has recently appeared which has involved the use of these materials as a permanent source of nutrition in patients following massive resection of the small intestine. An adequate state of nutrition has been maintained in one patient for more than 2 years (Jeejeebhoy, 1973) but the eventual application of this type of parenteral nutrition must await very careful assessment.

University Surgical Division and
Academic Division of Medicine,
University of Sheffield

R.G.CLARK, S.S.BLEEHEN
AND I.R.EDWARDS

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News and Notices

The Royal College of Pathologists

A two-day Symposium on Drugs and Disease organized by the Royal College of Pathologists will be held at the Royal College of Physicians, London N.W.1 on 6 and 7 February 1975.

Details and application forms may be obtained from The Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y 5AF.

APPOINTMENTS

The Middlesex Hospital and St John's Hospital for Diseases of the Skin

Dr Gerald Max Levene has been appointed Consultant Dermatologist to the Middlesex Hospital and St John's Hospital for Diseases of the Skin, London. Dr Levene qualified M.B., B.S. (Lond.) in 1959 and became M.R.C.P. (Lond.) 1963. He trained at the Royal Free Hospital School of Medicine, London and later became Registrar in the Connaught Hospital, London, Senior House Officer at the Royal Free Hospital, Senior House Officer at St John's Hospital for Diseases of the Skin, Registrar and Senior Registrar at St Thomas' Hospital, London. More recently he has been Senior Lecturer and Honorary Consultant Dermatologist at St John's Hospital and at the Royal Post-Graduate Medical School. He spent a year in the Department of Dermatology, Western Reserve University, Cleveland, Ohio, U.S.A. His special interests include the role of immunological mechanisms in the production of skin diseases and the use of immunosuppressive and cytotoxic agents in the treatment of skin disease; he has numerous publications on these subjects. He has recently published, as joint author, a highly successful colour atlas of dermatology.

The Institute of Dermatology, University of London

Dr Malcolm Watson Greaves has been appointed to the Chair of Dermatology, at the Institute of Dermatology, University of London. Dr Greaves qualified M.B., B.S. at Charing Cross Hospital Medical School in 1957 and, after several appointments in general medicine and paediatrics, obtained the M.R.C.P. (Lond.) in 1961. Following a registrarship in dermatology at St John's Hospital for Diseases of the Skin he was awarded an MRC Senior Clinical Research Fellowship which he held until 1966 in the Department of Pharmacology, University College London. In 1964 he graduated M.D. (Lond.) and in 1967, Ph.D. (Lond.) following a period of basic research on pharmacological aspects of skin disease. In 1966 Dr Greaves was appointed lecturer in dermatology in the University Department of Dermatology, Newcastle-upon-Tyne. He was appointed Senior Lecturer and Honorary Consultant Dermatologist in 1969 and Reader in Dermatology in 1973. He has continued and developed his research mainly in the field of pharmacological mediators of inflammatory skin disorders.

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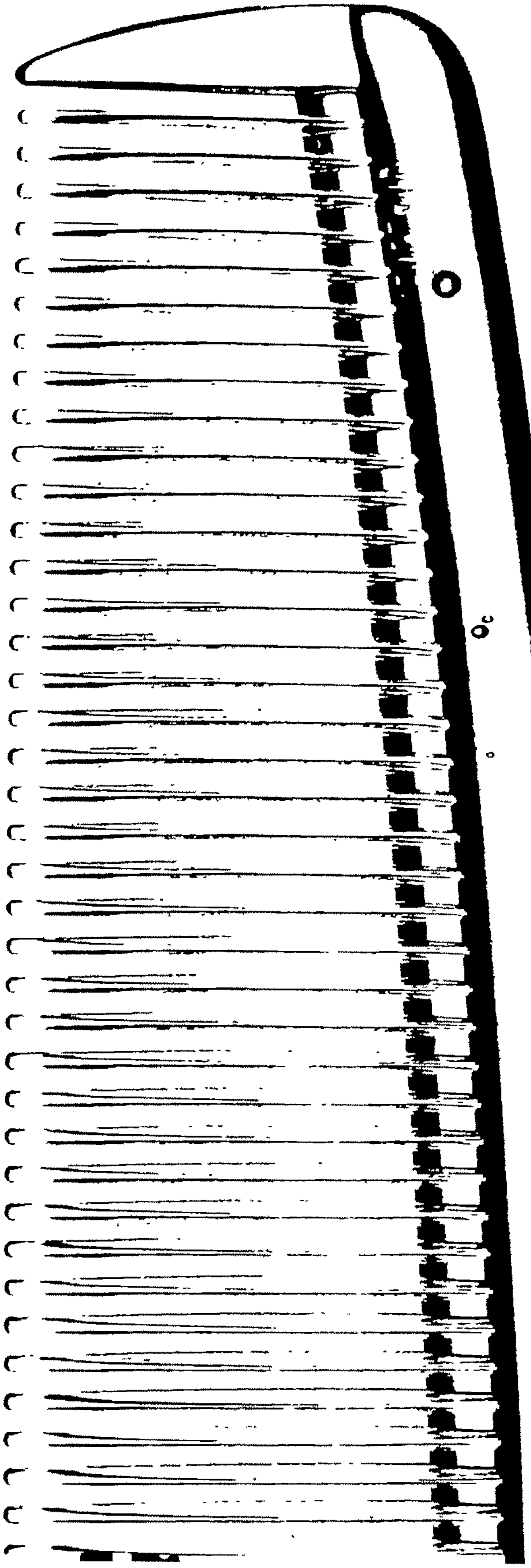

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Textbook of Dermatology

Edited by

Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Robert Harold Champion M.A. M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D.(Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Niels Hjorth M.D.(Copenhagen)
William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
Francisco Kerdel-Vegas M.D.(Caracas) M.SC.(N.Y.)
Gunnar Lumbolt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
Stephen Owen Bellamy Roberts M.A. M.B. B.CHIR.(Cantab.) M.R.C.P.(Lond.)
Arthur James Rook M.D.(Cantab.) F.R.C.P.(Lond.)
Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
Peter Derrick Samman M.A. M.D.(Cantab.) F.R.C.P.(Lond.)
Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
Ian Bruce Sneddon M.B. CH.B.(Sheffield) F.R.C.P.(Lond.)
Darrell Sheldon Wilkinson M.D.(Lond.) F.R.C.P.(Lond.)
Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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Foreword

As in 1973, this special supplement contains summaries of the papers and details of the cases to be presented at the fifty-fourth annual meeting of the British Association of Dermatologists. Those attending the meeting are asked to bring with them this supplement, which is also being distributed free to all subscribers to the journal thanks once again to the generosity of Glaxo Laboratories Limited.

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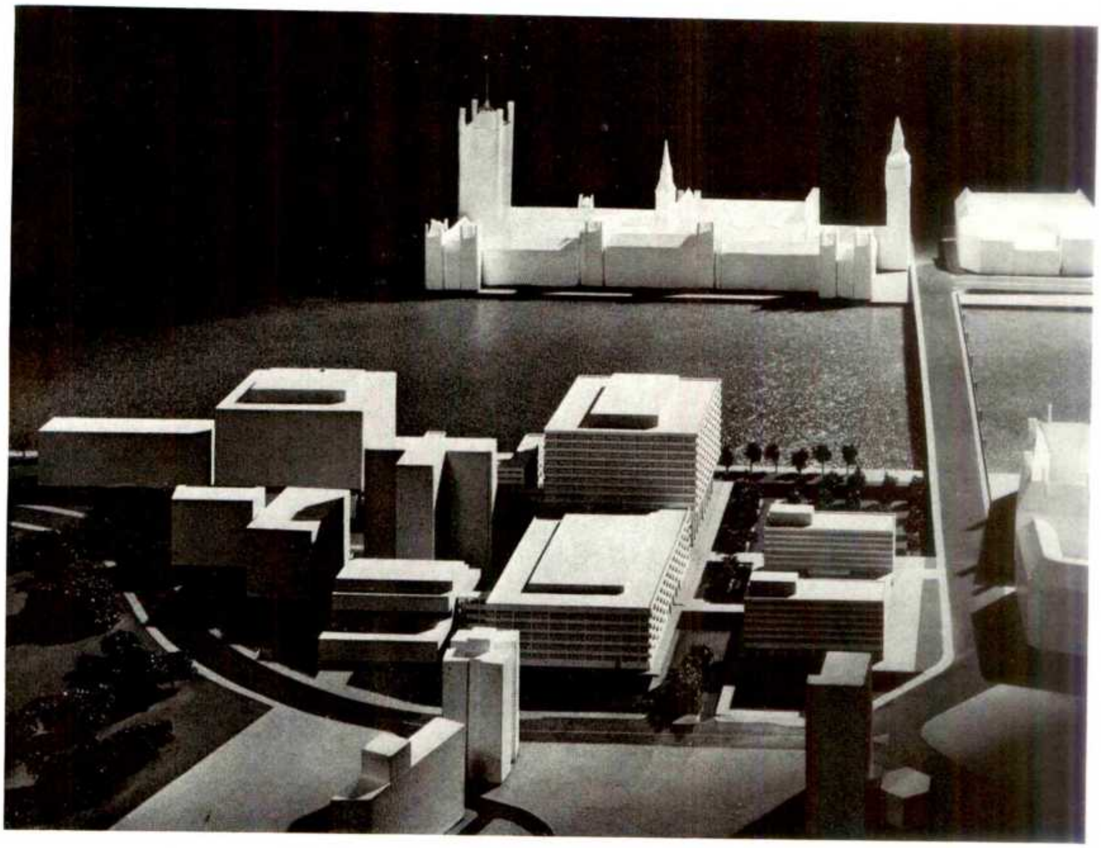
HUGH WALLACE

PRESIDENT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS, 1973–4.



THE NEW ST THOMAS' HOSPITAL, LONDON.

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British Journal of Dermatology (1974) **91,** Supplement **10,** 4.

British Association of Dermatologists Fifty-fourth Annual Meeting, 1974 and Franco-British Reunion

PRESIDENT: DR H.J. WALLACE

On Wednesday 3 July and Thursday 4 July, the meetings will be held at the Royal College of Physicians, Regent's Park. Friday 5 July is the day of the Franco-British Reunion and this will also be held at the Royal College of Physicians. On Saturday 6 July the Clinical Meeting will be in St Thomas' Hospital, London, S.E.1.

PROGRAMME OF MEETING

Wednesday 3 July

09.00	Registration	
09.15	Annual General Meeting (Members only)	
10.15	**Coffee**	
10.40	Rodney Dawber	Occupational koilonychia
10.55	J. Kirby, K. W. Heaton and J. L. Burton	The pruritic effect of bile salts
11.15	Louis Forman and Fergal F. Nally	Oral acquired non-dystrophic epidermolysis bullosa
11.30	Ian Sneddon and Joan Sneddon	What happens to patients with artefacts?
11.50	Ronald Church and John Knowelden	Scabies in the city of Sheffield, 1972–73
12.10	S. Zaynoun, Irene Hall, B. E. Johnson and W. Frain-Bell	A study of bergamot photosensitivity

12.30	**Lunch**	
14.00	L. Stankler	The effect of active and convalescent serum on the Köbner reaction in patients with active psoriasis
14.20	A. P. Warin, J. W. Landells, G. M. Levene and H. Baker	Serial liver biopsy findings in methotrexate-treated psoriasis
14.40	M. M. Black and W. L. Epstein	Formation of multinucleate giant cells in tuberculoid granulomas
15.00	N. R. Rowell	The prognosis of systemic sclerosis
15.20	Warwick L. Morison	Survey of viral warts, herpes zoster and herpes simplex in patients with secondary immune deficiencies and neoplasms
15.40	**Tea**	
16.10	F. Ray Bettley and Alan O'Shea	Arsenic retention in relation to carcinoma
16.30	B. Nissen and R. Marks	Eruptive syringoma, A clinico-pathological study of thirty-five patients
16.50	P. W. M. Copeman	Livedo reticularis—a clinical sign not a disease

Thursday 4 July

09.00	Registration	
09.10	M. Williams, W. J. Cunliffe, D. Perera and P. Thackray	An explanation for the localization of acne
09.30	S. K. Goolamali and S. Shuster	A sebotrophic stimulus in benign and malignant breast tumours
09.50	H. Barnes, P. M. Gaylarde, A. P. Brock and I. Sarkany	An improved method of assessing topical corticosteroid activity
10.10	J. L. Burton, W. J. Cunliffe, G. Holti and V. Wright	Complications of topical corticosteroid therapy in patients with liver disease

10.30	**Coffee**	
11.00	T. B. Fitzpatrick, K. Jimbow and D. D. Donaldson	Dominant oculo-cutaneous albinism
11.20	A. N. Okoro	Albinism in Nigeria: a clinical and sociological study
11.40	Rona M. MacKie, A. J. Cochran, R. M. Grant, C. E. Ross and D. E. Hoyle	Malignant melanoma; a correlation between histological features and tumour-directed cell-mediated immunity
12.00	J. A. A. Hunter and W. D. Paterson	Human malignant melanoma and the ultrastructural DOPA reaction
12.30	**Lunch**	
13.30	Coach leaves the Royal College of Physicians for Kew Gardens	
14.30	The Director of the Royal Botanic Gardens, Professor Heslop Harrison, F.R.S., has kindly agreed to talk on some aspect of pollen allergy	
15.30	**Tea** in the Royal Botanic Gardens and a walk in the Gardens for those who wish	
16.30	Coach will leave Kew Gardens and arrive at the Royal College of Physicians at 17.00 hours	
The Dowling–McGaw Golf Competition	To be played at the Roehampton Golf Club, Roehampton Lane, London, S.W.15 at 14.30 hours	
The Bowers-Sneddon Sailing Trophy	It is hoped that the competition will take place at the Welsh Harp, North Circular Road, N.W.10 at 14.30 hours	
18.30–20.30	President's Reception at the House of Lords	

Friday 5 July

Franco–British Reunion

09.00	Registration
09.15	Papers

The French papers are:

A. Bazex, J. Bazex, F. Broussy et D. Balas	Etude ultra-structurale d'un élément de Prurigo strophulus à la phase de début
J. Civatte et S. Belaich	Etude anatomo-pathologique des lesions papulo-pustuleuses (aphtes cutanés) au cours de syndrome du Behçet
P. Laugier, F. Paccaud, M. Orusco, N. Hunziker and M. Monnard (Geneva)	Diagnosis of herpes: comparative virological studies
L. Schnitzler, B. Schubert, P. Belperron et J. L. Verret	Actinoreticulose survenant au decours d'une porphyrie cutanée tardive
J. Thivolet	Confrontations cliniques et immunopathologiques dans le lupus érythémateux
R. Touraine, G. Lagrue, J. Revuz, Mlle Hirbec, Mme Intrator et J. P. Farcet	Complément total, fractions C3, C4 et ponction biopsie rénale dans les connectivites dermatologiques sans atteinte rénale fonctionnelle

The British papers are:

J. A. Cotterill, L. Millard, M. M. Roberts, R. A. Forster and J. Sikorski.	Swimming-pool alopecia
M. S. R. Hutt	*Mycobacterium ulcerans* infection
Janet Marks, Sam Shuster, Sandra Young, R. H. Felix and A. J. Watson	The skin-gut relationship in dermatitis herpetiformis
R. S.-H. Tan, C. M. Butterworth, H. McLaughlin, Samuel Malka and P. D. Samman	Mycosis fungoides: a disease of antigen persistence
I. W. Whimster	The pathology of lymphangioma circumscriptum
E. Wilson Jones and K. Grice	Reticulate pigmented anomaly of the flexures (Dowling, Degos): a new genodermatosis

Saturday 6 July

09.30	Patients may be seen in Gassiot House, St Thomas' Hospital
10.30	**Coffee**
11.00	Discussion in the Nevin Lecture Theatre, East Wing, St Thomas' Hospital
12.30	**Lunch** in St Thomas' House
13.30	Coaches will leave St Thomas' Hospital to visit Sutton Place, the house of Mr Paul Getty. This will be followed by tea at the Civic Hall, Guildford
19.30 for 20.00	**Dinner** at the Tate Gallery, Whistler Restaurant

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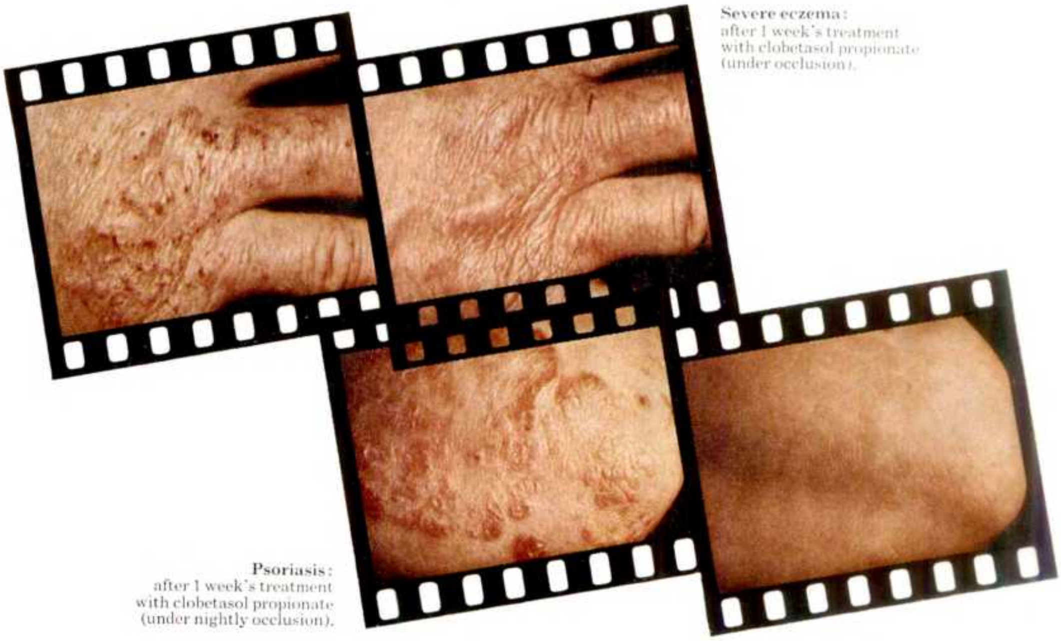

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British Journal of Dermatology (1974) **91,** Supplement **10,** 11.

SUMMARIES OF PAPERS

Occupational koilonychia

RODNEY DAWBER

Department of Dermatology, North Staffs Hospital Centre, Stoke-on-Trent

Koilonychia is most commonly seen as a sign of iron deficiency anaemia. It also occurs as an isolated congenital anomaly, as a temporary normal phenomenon in young children and in association with Raynaud's phenomenon (Samman, 1972).

Two motor mechanics presented to the Skin Department with koilonychia of fingernails which they attributed to contact with motor oils. Neither patient showed clinical or laboratory evidence of any underlying disease process.

In an attempt to confirm or refute this association, eight major motor vehicle service centres were visited; the nails of 174 employees were examined, ninety-four motor vehicle mechanics and eighty controls such as paint sprayers, panel beaters, etc. Koilonychia was observed in five motor mechanics but in none of the controls. In view of previous reports that koilonychia may be due to deficiency of sulphur-containing amino acids, nail keratin amino acids analysis was carried out. No significant difference was found compared with normal controls.

Occupational koilonychia is thus not uncommon in motor mechanics, possibly due to contact with motor oils. The results of amino acid analysis suggest a different pathogenesis to other types of koilonychia.

REFERENCE

SAMMAN, P.D. (1972) *The Nails in Disease,* 2nd edn, p. 103. Heinemann, London.

The pruritic effect of bile salts

J.KIRBY,* K.W.HEATON AND J.L.BURTON

Departments of Dermatology and Medicine, Bristol Royal Infirmary

The pruritus of obstructive jaundice is thought to be due to bile salt retention. The correlation between serum bile salt concentration and pruritus is poor however (Osborn *et al.*, 1955), and so far as we know, the effect of the individual bile salts on the skin has never been tested.

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* Present address: Dermatology Department, St Bartholomew's Hospital, London.

We have now tested the sodium salts of the three main bile acids (cholic, deoxycholic and chenodeoxycholic) and their taurine and glycine conjugates (10 mM, conc.) in twenty-four subjects with localized non-itchy dermatoses. The solutions were tested by prick tests, 48 h patch tests, and by applying them to cantharadin blister bases on forearm skin (Arthur & Shelley, 1955). The results were compared with the effect of papain (16–40 BAEE units/mg, diluted 1:10) and normal saline.

The bile salts produced no reaction by prick testing or patch testing, but all of them produced itch in some, but not all, subjects when they were applied to a blister base. The various bile salts differed considerably in their tendency to produce pruritus. The most effective, chenodeoxycholate, produced pruritus in a significantly greater number of subjects than the least effective bile salt, cholate.

REFERENCES

ARTHUR, R.P. & SHELLEY, W.B. (1955) The role of proteolytic enzymes in the production of pruritus in man. *Journal of Investigative Dermatology*, **25**, 341.

OSBORN, E.C., WOOTTON, I.D.P., DA SILVA, L.C. & Sherlock, S. (1959) Serum-bile-acid levels in liver disease. *Lancet*, **ii**, 1049.

Oral acquired non-dystrophic epidermolysis bullosa

DR LOUIS FORMAN AND DR FERGAL F. NALLY

Department of Oral Medicine, Eastman Dental Hospital, London

Fourteen patients with recurrent blistering in the mouth have been observed for 1–5 years, in whom the obvious provocative factor was trauma. The area of maximal involvement was the labial gingivae. The patients presented with thick-roofed blisters together with denuded and bleeding areas with detached borders of epithelium, or with defined red boggy areas of mucosa. Re-epithelialization of the raw areas usually occurred within a week. No atrophy, shrinkage, or epithelial bridges were observed. The blistering has been confined to the mouth in all cases; the most striking feature was the extreme fragility of the epithelium.

The blisters would occur spontaneously or could easily be produced by gentle friction on normal-looking mucosa, and then tended to fill with blood. In addition, a blunt probe could be inserted under the edge of a ruptured bulla (the probe test) to the full extent of the instrument (1½ cm). A sub-epithelial bulla was found on biopsy but no circulating epithelial antibodies could be demonstrated.

Healing occurred without scar formation. The disease occurred in middle-aged patients (average age 52 years) and there was no family history (with one exception). In some patients the condition gradually disappeared and as it did so the probe test became more difficult to demonstrate. Because of the fragility of the epithelium the term 'acquired oral non-dystrophic epidermolysis bullosa' is suggested.

What happens to patients with artefacts?

DR IAN SNEDDON AND DR JOAN SNEDDON

Rupert Hallam Department of Dermatology, Sheffield

A study of forty-one patients suffering from dermatitis artefacta seen in the last 15 years has been made. Examples of the type of lesions and their histories will be given.

Of the forty-one patients, all but four were female, and the majority were young girls, but eight were women over 40 years old.

Most patients were investigated by social workers and psychiatrists, but many refused psychiatric help at the time they were suffering from the skin artefacts. The difficulty in persuading such patients to see a psychiatrist led to considerable problems in management.

In the young girls, the motive for production of the artefact was similar to the unsuccessful suicide attempt, 'a cry for help.' A dominant and aggressive mother was frequently in the background and the girls tended to be mentally and sexually immature, comparable to those suffering from anorexia nervosa.

A long-term follow-up of the more severe cases has shown that the artefact was only one facet of a series of episodes which included simulated pyrexia, hysterical aphonia, paraplegia and self-poisoning attempts. Only one patient has successfully committed suicide. Many of this severe group have had multiple admissions to psychiatric wards, and most of the severe cases were still suffering from psychiatric symptoms 10–15 years after the diagnosis of artefact had been made.

Scabies in the city of Sheffield, 1972–73

RONALD CHURCH AND JOHN KNOWELDEN

Rupert Hallam Department of Dermatology, Sheffield

For several years a system of voluntary notification of scabies by the general practitioners, to the Medical Officer of Health, has been in operation; and the total number seen in the skin department, or notified, varied from 100–150 cases annually. In June 1973 general practitioners were invited to refer cases suspected to be suffering from scabies to hospital. Those contacts of the confirmed cases, who were suspected to have skin disease, were then traced to their homes by a specially trained state registered nurse.

From June 1972 to June 1973, 1482 patients with confirmed scabies were either referred or traced and of these, 609 were thought to be the primary case in a household. Where the source of infection in the primary cases could be found, relatives from outside the household accounted for 12% and friends for 23%. Contacts at school were surprisingly insignificant at 3%. The age group 10–19 accounted for the greatest number of primary cases. In this age group females predominated and in the main they acquired the disease from members of their own sex.

Mapping the cases within the city boundary showed that the disease was disseminated throughout the city with a concentration in the areas of lower socio-economic status.

The disease could be traced through large family groups occupying different households, and these appear to be responsible for the major part of the spread of the disease; either spread by contact at school or by venereal contact, the latter playing an insignificant part.

It is concluded that inadequate treatment of all members of an infected household is the main cause of the lack of control of the disease.

A study of bergamot photosensitivity

DR S.ZAYNOUN, IRENE HALL, DR B.E.JOHNSON AND DR W. FRAIN-BELL

Department of Dermatology, University of Dundee

Pigmentation of the skin of the face and neck has been attributed to the interaction between a photoactive chemical and ultraviolet irradiation. Current dermatological texts do not clearly define the investigative procedures which should be used and we wish to report a study of the available techniques.

Method. (*a*) *In vivo.* In a group of ninety-three subjects (twelve normal controls, seventy-eight with photodermatoses, and three with melanoderma) photopatch tests were carried out for two separate samples of oil of bergamot obtained from different commercial sources (A and B) with a concentration range of 1–20% in soft paraffin (PMF) or ethanol as the vehicle. Irradiation was provided by a black light fluorescent tube (Philips TWL 20) with an emission spectrum of 300– 350–400 nm, filtered to remove the short U.V. wavelengths <320 nm. A comparison was made between oil of bergamot samples A and B; between PMF and ethanol as the vehicle; between open and closed patch tests; between irradiation at 1 h after the application of the test substance and at hourly intervals up to 8 h; and between irradiation after one application and a range of multiple daily applications.

(*b*) *In vitro.* Bergamot oil samples A and B were fractionated using thin layer chromatography; the fractions obtained were added to *Candida albicans* cultures and exposed to black light, long u.v. irradiation, to determine which was the responsible photo-active fraction.

(*c*) *In vivo.* The photoactive fractions obtained from the *in vitro* studies (b) were used for photopatch testing as in (a).

Results. Using 10% bergamot oil in PMF, positive photopatch tests were obtained in nineteen out of the sixty-five photodermatoses subjects when using sample A (30%) and in only one out of twenty-three subjects with sample B (5%). In the twelve normal subjects, using sample A with a 24 h closed patch test, a positive response was obtained in 17% with a PMF vehicle and 50% with ethanol. However, when the open test technique was used a 90% positive response was obtained with PMF and 75% with the ethanol vehicle.

Positive responses also tended not to occur when the irradiation was postponed for 8 h or longer, but to be more marked if preceded by a number of daily applications of the bergamot oil. Of the three subjects with melanoderma investigated during 1972, before sample A became available, a faint positive response was obtained in only one, that being the most severely affected patient. A positive reaction, however, was obtained when sample A subsequently became available in one of the two remaining cases. *In vitro* studies showed that one fraction only was responsible and this was confirmed by obtaining positive photopatch tests in human subjects. This fraction was also found to be present in only minimal amounts in the bergamot oil specimen B.

These results highlight the importance of using a bergamot sample which contains adequate amounts of the photo-active fraction; and of appreciating that a phototoxic response would be obtained in any individual, provided an open test technique is used with sufficient concentration. Therefore, in the study of the relevance of bergamot oil photosensitivity, in cases of melanoderma and photodermatoses, the significance of positive photopatch test results can only be determined by comparison with the results obtained using the same concentrations, vehicles, and test techniques, in normal control subjects.

The effect of active and convalescent serum on the Köbner reaction in patients with active psoriasis

L. STANKLER

Department of Dermatology, Aberdeen Royal Infirmary, Aberdeen

Eight patients with psoriasis took part in the investigation. Blood was taken from these patients when the disease was active and again in the convalescent stage when all the lesions had disappeared. The active and convalescent sera, from the blood samples, were stored in the deep freeze at −20°C and used on these same patients when their psoriasis became active again. The skin over the front of the forearm was scraped to bleeding, over an area about 8 mm in diameter, at three different sites. Then 0.2 ml of autogenous active serum, or of convalescent serum, was injected into the dermis at two of the scraped sites; then a similar amount of sterile normal saline into the third site, as a control.

The following results were obtained. In five patients psoriasis developed at scraped sites injected with saline or active serum, but not at the sites injected with convalescent serum. In one patient all the lesions became infected and developed psoriasis. The remaining two patients did not develop psoriasis at any of the scraped sites.

It is concluded that the serum of patients recovering from active psoriasis contains a factor which inhibits the development of psoriasis.

Serial liver biopsy findings in methotrexate-treated psoriasis

A.P.WARIN, J.W.LANDELLS, G.M.LEVENE AND H.BAKER

The London Hospital, and St John's Hospital for Diseases of the skin, London

We have previously reported our retrospective study of the effects of methotrexate, when used for psoriasis, on hepatic structure and function (Almeyda *et al.*, 1972). The purpose of this preliminary communication is to transmit the results to date of our prospective study of liver biopsy findings in

TABLE 1. Inflammatory cells, fibrosis and cumulative methotrexate dosage in serial liver biopsies

Case	Age now (years)	Pre-treatment biopsy		Serial biopsies during treatment											
				First			Second			Third			Fourth		
		Cells	Fibrosis	Cells	Fibrosis	Cumulative dose (g)	Cells	Fibrosis	Cumulative dose (g)	Cells	Fibrosis	Cumulative dose (g)	Cells	Fibrosis	Cumulative dose (g)
Mrs S.H.	24	0	0	0	0	0·39	0	½	0·63	0	0	1·7	—	—	—
Mr F.O'K.	58	½	0	0	0	0·89	1	0	1·54	—	—	—	—	—	—
M A.S.	38	1	0	0	0	—	0	0	1·61	—	—	—	—	—	—
Mr. W.P.	43	½	0	0	0	0·3	0	0	0·85	—	—	—	—	—	—
Mr W.C.	41	0	0	0	0	1·02	½	0	2·0	—	—	—	—	—	—
Mrs H.W.	53	½	0	0	0	0·61	0	0	0·61	—	—	—	—	—	—
Mrs L.F.	27	0	0	0	0	0·66	0	0	1·11	—	—	—	—	—	—
Mrs M.H.	40	0	0	0	0	0·28	1	0	0·76	0	0	1·18	—	—	—
Mrs M.S.	54	½	0	0	0	—	0	0	—	0	0	—	0	0	3·1
Miss G.Y.	65	0	0	0	0	0·17	0	0	0·17	—	—	—	—	—	—
Mr A.C.	66	0	0	0	½	1·47	—	—	—	—	—	—	—	—	—
Mr O.B.	53	0	0	1	1	—	½	0	2·25	0	0	2·85	½	½	3·68

0 = Nil, ½ = very mild; 1 = mild; 2 = moderate; 3 = severe.

twelve patients studied before and approximately annually after the commencement of methotrexate, using only weekly dosage schedules. There were equal numbers of women and men, whose ages ranged from 24 to 66 (mean 45 years); only one had been a heavy drinker.

The presence of inflammatory cells and of fibrosis were each recorded by J.W. Landells on a scale from 0 (nil), through ½ (very mild), 1 (mild) and 2 (moderate) to 3 (severe). Cirrhosis was either present or absent.

Methotrexate has been given for periods ranging from 19 to 47 months; weekly dosage has never exceeded 0·4 mg/kg body weight. The pre-methotrexate findings are shown in Table 1 and reveal that no patient had significant liver damage. Two patients had four subsequent biopsies; two had three; seven had two and one had only one. The pathological findings (with the cumulative dosages at each biopsy) are also shown in Table 1.

No patient has developed cirrhosis or grades 3 or 2 of fibrosis. One showed grade 1 fibrosis at the first post-treatment biopsy, but was grade 0 at the second and third (2.85 g methotrexate given) and grade ½ at the fourth biopsy after 43 months (3·68 g). Two others showed fibrosis grade ½ after 0·63 g and 1·47 g respectively, but the former of these was again grade 0 after 34 months (1·7 g). No patient has developed grades 3 or 2 of inflammatory cells. Two showed grade 1 changes, but in both these cases they disappeared in subsequent biopsies.

Conclusion. In this small series of patients our prospective study has as yet shown no evidence of significant, let alone progressive, hepatic structural damage from methotrexate in weekly dosage. These results are in keeping with our findings on a larger number of patients who were also only treated by weekly dosage and subjected to serial biopsy; they are not included here because pre-methotrexate liver biopsies (before 1969) were not performed.

Secondly, the study has revealed fluctuation in the mild degrees of inflammation or fibrosis observed. It is not known whether these fluctuations are due to observer error, sampling error caused by focal pathological changes or reflect truly fluctuating events.

It is suggested that some of the hepatotoxicity reported earlier, by ourselves and others, may be attributable to the now abandoned daily dose schedules and failure to recognize pre-methotrexate liver damage.

REFERENCE

ALMEYDA, J., BARNARDO, D., BAKER, H., LEVENE, G.M. & LANDELLS, J.W. (1972) Structural and functional abnormalities of the liver in psoriasis, before and during methotrexate therapy. *British Journal of Dermatology*, 87, 623.

Formation of multinucleate giant cells in tuberculoid granulomas

M.M.BLACK AND W.L.EPSTEIN

Department of Dermatology, School of Medicine, University of California, San Francisco

Multinucleate giant cells (MGC) are a characteristic cellular component of tuberculoid granulomas but previously the histogenesis of MGC has not been thoroughly investigated. Tuberculoid granulomas were produced experimentally by injecting intradermally dilute suspensions of zirconium and beryllium salts into twenty-six individuals who had been previously sensitized to these metals. More than 100 biopsies were obtained at intervals of between 5 days and 13 months later and the specimens fixed and prepared for light microscopy. TdR-H^3 was injected into thirty-two granulomas at various stages in their evolution and biopsies taken from 40 min to 2 or more weeks later. Eighteen biopsies were processed for electronmicroscopy and sixty-four MGC were examined ultrastructurally. MGC occurred commonly, both within organized tubercles and adjacent to necrotic areas. MGC cytoplasm was highly distinctive, and very different from typical epithelioid cells (secretory EC) or undifferentiated mononuclear cells. The characteristic hallmark of these MGC was the presence of myriads of mitochondria adjacent to nuclei with many membrane-lined vesicles in the centre of the cell. Their occurred mainly in disrupted oedematous tubercles in which the epithelioid cells had cytoplasmic features similar to those of the MGC (vesicular EC). Fusion of the membranes appeared to occur between these epithelioid cells having similar cytoplasmic features. No nuclear labels within MGC occurred in any of the 40 min specimens, indicating that normal nuclear division does not occur within giant cells.

We postulate that in damaged and necrotic areas epithelioid cells cease their normal secretory function (secretory EC), accumulate mitochondria and vesicles (vesicular EC) and then fuse to form MGC.

The prognosis of systemic sclerosis

N.R.ROWELL

General Infirmary, Leeds

From a personal series of eighty-one patients with systemic sclerosis evidence is produced to indicate that the disease process is speeded up, and the prognosis is poorer, in males as compared with females.

The prognosis in any particular patient is unpredictable. Fulminating onset with gangrene can be followed by survival for many years, yet other patients may die within a year of onset.

Prognosis is not related to the presence or absence of antinuclear antibodies or to treatment.

The so-called CRST syndrome is not a separate entity. These features can be associated with severe systemic abnormalities.

Survey of viral warts, herpes zoster and herpes simplex in patients with secondary immune deficiencies and neoplasms

DR WARWICK L. MORISON

St Helier Hospital, Carshalton

The main aim of the investigation was to determine whether there is a different incidence of viral warts in patients with cell-mediated immune deficiency as compared to patients with humoral immune deficiency. Three hundred and ninety-seven patients with secondary immune deficiency were compared with 348 control subjects. The occurrence rates for warts are shown in parentheses:

Hodgkin's lymphoma (25·7% of patients examined) as an example of predominant cell-mediated immune deficiency.

Multiple myeloma (0%) as an example of predominant antibody deficiency.

Chronic lymphatic leukaemia (17·6%) as an example of mixed antibody and cell-mediated immune deficiency.

Control subjects (2·3%).

The low incidence in patients with antibody deficiency would indicate that the humoral immune system has little influence on the natural history of warts. The greatly increased incidence in patients with cell-mediated immune deficiency would indicate that this immune mechanism has considerable influence on the incidence of warts and their subsequent resolution.

For comparison, the incidence of past herpes zoster and herpes simplex infections was also investigated in the same patients with the following results:

Hodgkin's lymphoma	H. zoster 26·9%	H. simplex 39·1%
Multiple myeloma	H. zoster 15·3%	H. simplex 20·5%
Chronic lymphatic leukemia	H. zoster 27·5%	H. simplex 39·2%
Control subjects	H. zoster 10·9%	H. simplex 39·0%

In addition the incidence of the same three viral infections was investigated in 236 patients with cutaneous and systemic neoplasms:

Viral warts 5·1%
Herpes zoster 13·5%
Herpes simplex 27·1%

Arsenic retention in relation to carcinoma

F. Ray Bettley and Alan O'Shea

The Middlesex Hospital, London

Several authors have commented that in the production of arsenical carcinoma and keratoses an individual predisposition seems to play some part.

In the present investigation patients with arsenical carcinomas were given a standard dose of arsenic by mouth and the resulting blood levels and urinary excretion determined.

By comparison with normal subjects the test series showed significantly lower blood levels and lower excretion, indicating that more arsenic was fixed in the tissues.

This aberration in the handling or metabolism of arsenic may account partly or entirely for this apparent predisposition to arsenical carcinoma. If retention of arsenic is sufficiently pronounced, even slight exposure, for example in water or tobacco, might lead to skin carcinoma, the origin of which could escape detection.

Eruptive syringoma, a clinico-pathological study of thirty-five patients

B.NISSEN AND R. MARKS

Institute of Dermatology, London and The Welsh National School of Medicine,

The appearance of numerous lichenoid papules containing epithelium derived from eccrine sweat glands, distributed over the face, trunk and limbs is an unusual and fascinating clinical phenomenon. In this study all patients with this diagnosis who had presented at St John's Hospital for Diseases of the Skin in the past ten years, and in whom the diagnosis could be substantiated histopathologically, were reviewed. Thirty-five patients satisfied these criteria and form the basis of this report. The disorder was found to be very much more common in women and usually made its first appearance in the second, third and fourth decades. The lesions were often numerous but apart from their appearance did not cause any symptoms. Once present, there was no tendency for resolution and the lichenoid papules persisted unchanged for many years. Histopathologically the lesions presented a remarkably uniform appearance regardless of the age, sex or type of lesion. The only marked difference between samples taken from different lesions was the density of the abnormal epithelial structures.

The initial eruptive phase and the subsequent apparent total inactivity of the lesions pose interesting questions about the histogenesis and generative cycle of syringomata. To try to throw some light on these problems two lesions were examined enzyme histochemically. In addition two lesions were injected with tritiated thymidine in order to label cells in the premitotic DNA synthesis phase. Surprisingly the lesions are strongly reactive enzyme histochemically and the proportion of cells that became labelled autoradiographically was similar to that of normal eccrine secretory tissue.

Livedo reticularis—a clinical sign not a disease

DR P.W.M.COPEMAN

Westminster Hospital, London

Livedo reticularis is frequently a valuable presenting sign or diagnostic adjuvant of grave systemic disease. In itself it is not a complete diagnosis. Nonetheless it is often equated popularly with 'connective tissue' diseases, as though it is pathognomonic of the condition.

The sign can be explained mechanistically on the basis of (1) the normal anatomical disposition of cutaneous blood vessels, and (2) the natural rheological properties of whole blood; its increased viscosity at low temperatures and in certain diseases.

Livedo reticularis has various anatomical origins in disease, one or more of which can be relevant in the patient.

(1) Pathological vessels. (a) Arterioles: obstruction to the inflow of blood. (b) Capillaries: hyperviscosity of the blood flowing within. (c) Collecting venules: obstruction to the outflow of blood.

(2) Normal vessels: heat transfer deficiencies.

The conditions underlying these disorders include the following:

(1) (a) Inflow defect: arteriosclerosis, emboli, polyarteritis nodosa, systemic lupus erythematosus, rheumatoid arthritis, syphilis. (b) Blood hyperviscosity: solid elements (polycythaemia, thrombocythaemia); plasma (cryoprecipitable or macro proteins), the capillary-venular localization of the

pathology is caused by sludging. (c) Outflow defect: immunological as in cutaneous angiitis (venulitis), haemodynamic back pressure aggravated by heart failure and dependency.
(2) Blood reaches the skin in a patchwork pattern; round zones of skin are supplied by central arterial cones of fast flowing blood and each zone drains peripherally into a network (reticulate) system of collecting venules. These drainage venules are splayed out horizontally, close beneath the epidermis where they are vulnerable to surface temperature. The sluggish venous blood flow, in health, shows itself as livid (deoxygenated) blood; this sign is accentuated in the cold due to the normal increase of whole blood viscosity with greater transit time.

The main damage inflicted by temperature extremes is upon the venules because heat and cold are flushed away by the circulating blood less speedily. The direct thermal damage to the adjacent tissues and to the vessels themselves causes intravascular stasis and clotting which will show as inflammation, ulcers, scars, and post-inflammatory hyperpigmentation. Cold (chilblains) or heat (erythema ab igne) induced disease spares the arterial central zones where the fast blood flow allows a speedier temperature equilibration.

An explanation for the localization of acne

M.WILLIAMS, W.J.CUNLIFFE, D.PERERA AND P.THACKRAY

General Infirmary, Leeds

There are several possibilities as to why acne is predominantly localized to the face; these possibilities include a variation both in the number of pilo-sebaceous ducts and in the size of the duct orifice. The duct exit is the final route through which sebum must pass; the smaller the orifice, the greater the outflow resistance and the greater the risk of acne.

We therefore measured these two variables using surface microscopy in 150 subjects. The pilo-sebaceous units on the forehead were significantly more numerous ($P = 0{\cdot}001$) than on the back and these in turn were more numerous than on the chest ($P = 0{\cdot}001$). The pilo-sebaceous exits on the forehead were significantly smaller than on the back ($P = 0{\cdot}001$) and the ducts on the back had a smaller orifice than those on the chest ($P = 0{\cdot}001$). Acne is more prevalent on the lateral part of the back where the pore size was significantly smaller than mid-line ducts ($P = 0{\cdot}01$). There was also a relationship between the severity of the acne and the size of the pilo-sebaceous duct.

We therefore conclude that the number of pilo-sebaceous units and the pilo-sebaceous exit size are important factors in the localization of acne.

A sebotrophic stimulus in benign and malignant breast tumours

S.K.GOOLAMALI AND S.SHUSTER

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne

Sebum excretion is increased in patients with breast cancer but the mechanism is unknown. We measured the sebum excretion rate (SER) in thirty-four patients with breast cancer and in forty-nine patients with bengin breast tumours; first before, and then after treatment by surgery and in some cases deep X-ray.

The SER was found to be significantly increased both in patients with breast cancer and in those with benign tumours as compared with age-matched controls. The raised SER was still apparent when sebum output was measured 6–12 weeks post-operatively, though there was a significant fall in a sub-group of eight patients in the 40–49 age group with cancer.

These results suggest that an increase in SER occurs both with benign and malignant breast tumours and this increase may fall post-operatively. The rise in SER is apparently not due to surgical stress because no increase in SER was found in patients in whom it was measured before surgery for conditions such as cholecystectomy varicose veins ligation.

These findings suggest that a specific sebotrophic stimulus is associated with benign and malignant breast tumours. The nature of this stimulus is uncertain.

An improved method of assessing topical corticosteroid activity

H.BARNES, P.M.GAYLARDE, A.P.BROCK AND I.SARKANY

Royal Free Hospital, London

Topical application of ointment bases causes varying degrees of epidermal thickening in guinea-pigs. This is reproducible and can be accurately measured. Suppression of these changes was produced by addition of betamethasone 17-valerate, fluocinolone acetonide and fluocinonide. The inhibitory effect of these steroids was related to the type of corticosteroid, its concentration and the ointment base. The effect was still evident at extremely high dilutions of the steroids and could be measured at concentrations as low as $5 \times 10^{-5}\%$ of fluocinonide in FAPG.

This technique has the merits of being reproducible, sensitive and accurate. It should find a place among existing methods in assaying the efficacy of topical corticosteroids and in aiding the selection of bases most suitable for formulation of these preparations.

Complications of topical corticosteroid therapy in patients with liver disease

J.L.BURTON,* W.J.CUNLIFFE,† G.HOLTI‡ AND V.WRIGHT§

**Department of Dermatology, Bristol Royal Infirmary, Bristol, †Department of Dermatology, The General Infirmary, Leeds, ‡Department of Dermatology, The Royal Victoria Infirmary, Newcastle-upon-Tyne, §Department of Rheumatology, The General Infirmary, Leeds*

Certain systemic complications of topical corticosteroids such as pituitary–adrenal suppression are well known. The clinical details of three patients will be reported; all had extensive skin disease unresponsive to topical tar or dithranol preparations. Consequently, these patients applied large amounts of topical steroids over large areas of skin for many years. The patients were also chronic alcoholics and had evidence of impaired liver function.

Two patients developed the clinical appearance of Cushing's syndrome and acute Addisonian crises when they failed to apply the topical steroids. Two patients also developed aseptic necrosis of weight bearing bones during their illness.

It is suggested that as a result of impaired liver function the steroids would be metabolized much more slowly and so produce a Cushingoid appearance. Aseptic necrosis can occur as a result of liver disease and of steroid therapy, and so it does not seem unreasonable to suggest that both factors were important in our patients. There is no doubt, however, that the Addisonian crises were entirely due to pituitary–adrenal suppression following topical corticosteroid application.

It is therefore important to be more aware of the systemic effects of topically applied corticosteroids in patients with liver disease.

Dominant oculo-cutaneous albinism

T.B.FITZPATRICK, K.JIMBOW AND D.D.DONALDSON

Departments of Dermatology and Ophthalmology, Harvard Medical School, Boston, Massachusetts

Albinism is a congenital heritable hypomelanosis that is limited to the eye (ocular albinism, an X-linked recessive trait) or that involves the eye, the skin and hair (oculo-cutaneous albinism, an autosomal recessive trait) (Fitzpatrick & Quevedo, 1972).

This report describes a new type of albinism in which there is a moderate dilution of skin and hair colour but the only eye change is iris translucency; there is no change in visual acuity as is invariably present in recessive oculo-cutaneous albinism. The propositus was a 5-year-old boy who had, since birth, marked intolerance to sunlight characterized by easy sunburning. The child had blond hair, pale blue eyes, and very fair skin which tanned only slightly. The only eye change was iris translucency: there was no nystagmus or decrease in visual acuity. Study of biopsy specimens of the exposed skin revealed the presence of melanocytes with a few partially melanized melanosomes, being indistinguishable from that observed in a fair Caucasoid skin. Fair skin, blond hair, decreased tolerance to sunlight, pale blue eyes, and iris translucency were present in the father, paternal uncle, and paternal grandmother, indicating a Mendelian dominant trait. Other members of the family including a paternal aunt and paternal grandmother had dark-brown hair, dark skin, brown eyes, and a marked ability to tan.

This heritable syndrome which is termed dominant oculo-cutaneous albinism (DOCA), provides an example of linkage between eye, skin and hair colour in which there are none of the serious visual defects that are present in recessive oculo-cutaneous albinism. It is proposed that individuals with blond hair, pale blue eyes, fair skin and a history of easy sunburning may, in fact, be examples of dominant oculo-cutaneous albinism, and eye examinations should be done for the presence of iris translucency.

Although the dominant inheritance of DOCA does not fit the polygenic system proposed for skin coloration in man (Quevedo, 1973), this syndrome does indicate that a coupled dilution of skin and eye colour may occur with both organs functioning within the normal range. The possible adaptive advantage of the diluted eye colour reported in this syndrome in a particular environmental setting remains to be demonstrated.

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Albinism in Nigeria: a clinical and sociological study

A.N.Okoro

General Hospital, Enugu, Nigeria

A study of 1000 albinos, all of Negro stock, showed various types of albinism with their different modes of transmission; these were oculo-cutaneous, ocular, cutaneous and piebald. The striking disparity between albino incidence among the nomadic communities and the settled communities is related to marriage habits and inbreeding tendencies. The greater the inbreeding tendency, the higher the albino incidence.

The sun and the society are hostile to the albinos. Under the merciless tropical sunshine, their melanin-deficient skin develops lentigines, actinic keratoses, wrinkles and epitheliomata from which they die in early adult life or in middle age at best.

Albinos are taunted from the cradle to the grave with such names as 'D.O.' (District Officer), a reference to the days when District Officers were Europeans.

Myopia and other ocular defects retard their progress in school and they eventually drop out to seek disastrous, menial outdoor occupations.

Registering albinos early in life, assuring their families that albino defects are confined to the skin and eyes, advising on protective clothing and sun-screening agents, correction of myopia, assistance with indoor occupations, and early treatment of actinic keratoses and skin cancer are enabling many albinos to attain their natural destiny and a ripe old age.

Malignant melanoma; a correlation between histological features and tumour-directed cell-mediated immunity

Rona M.MacKie, A.J.Cochran, R.M.Grant, C.E.Ross and D.E.Hoyle

University Departments of Dermatology and Pathology, Western Infirmary, Glasgow

A total of sixty-seven patients presenting with malignant melanoma have been assessed at the time of surgery for their primary lesion. No patient in this group had clinical signs of regional node involvement or of visceral metastases.

Tumour-directed cell-mediated immunity has been investigated in all patients using the leukocyte migration inhibition technique; antigen-mediated lymphocyte transformation and lymphocyte cytotoxicity to target tumour cells have also been assessed in a proportion of cases. Forty-seven of these patients (70%) have shown evidence of cell-mediated immunological activity against their melanoma-associated antigens, either homologous or autologous.

Histological features examined included tumour profile, predominant tumour cell type, presence of epidermal ulceration, histogenetic pattern, presence of tumour cells in lymphatic or vascular spaces, mitotic rate, and density and site of any tumour-associated inflammatory cell infiltrate.

Evidence of a tumour-directed cell-mediated immune response has been found most frequently in those individuals whose tumour has a high mitotic rate, and in which there is evidence of invasion of lymphatic or vascular channels. The other features studied occurred with equal frequency in the immunologically reactive and the non-reactive groups.

Human malignant melanoma and the ultrastructural DOPA reaction

DR J.A.A.HUNTER AND DR W.D.PATERSON

Department of Dermatology, University of Edinburgh, The Royal Infirmary, Edinburgh

Subcellular degrees of de-differentiation in malignant melanocytes are reflected by changes in the shape, size and pigment content of melanosomes. The purpose of this study was to determine whether such melanosomal alterations are associated with an abnormal ultrastructural DOPA reaction.

Materials. Tumour material was obtained from thirty patients at operation and fixed for routine electron microscopy (EM) and EM histochemistry. Ten specimens were selected for the EM DOPA reaction (Hunter, Mottaz & Zelickson, 1970), so that the series included a spectrum of melanosomal abnormalities.

Results. Numerous melanosomal structures were noted ranging from normal looking organelles to grossly abnormal granular forms. Abortive pre-melanosomal structures included those with melanofilament fragments and lamellar bodies. Melanosomal shape showed considerable variation and their size ranged from an average transverse diameter of 0·2–0·6 μm.

DOPA reacted product (RP) occurred only in tumour cells and was noted in all specimens. It was seen in Golgi saccules, the Golgi associated endoplasmic reticulum and Golgi vacuoles and vesicles. It was also seen in circular and horseshoe forms lining large vacuoles (up to 0·6 μm diameter) scattered throughout the cytoplasm. The RP pattern was similar in each tumour.

Subcellular de-differentiation, exhibited by alterations in melanosomal structure, is not reflected by changes in the localisation of DOPA oxidase activity.

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FRANCO-BRITISH REUNION

Etude ultra-structurale d'un élément de prurigo strophulus à la phase de début

(English translation below)

A.BAZEX, J.BAZEX, F.BROUSSY ET D.BALAS

En microscopie optique, on note, au niveau de l'épiderme l'existence d'une cavité intra-épidermique résultant des effects conjugués de la cytolyse et de l'acantolyse cellulaire. Dans cette cavité s'accumule de la sérosité et de rares cellules inflammatoires.

Au niveau du derme superficiel, il apparaît des microcavités, secondaires à des altérations des fibres conjonctives (mise en évidence par la technique de Gomori). Par ailleurs, le derme est le siège d'un important infiltrat péri-vasculaire polymorphe.

En microscopie électronique, on se rend compte que la cavité intra-épidermique résulte de l' acantholyse des cellules du corps muqueux de Malpighi qui sont en voie de cytolyse, et dont les desmosomes sont en partie détruits.

Au niveau du derme superficiel, on aperçoit une bulle multiloculaire résultant de la dégénérescence, puis de la lyse des fibres de collagène, élastiques et de réticuline. La membrane basale peu modifiée reste adhérente à l'épiderme. Autour de cette zone dégénérée, existe un infiltrat péri-vasculaire constitué de lymphocytes, d'histiocytes et surtout de polynucléaires de type eosinophile. La paroi des capillaires est peu modifiée.

Ces altérations dermo-épidermiques nous paraissent secondaires a l'injection dans la peau d'enzyme contenus dans la salive des insectes et sont totalement différentes des lésions de début de l'érythème polymorphe auxquelles on a souvent voulu les comparer.

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English translation

Ultrastructural study of the early stage of a lesion of papular urticaria

A. BAZEX, J. BAZEX, F. BROUSSY AND D. BALAS

With light microscopy one notes, at the level of the epidermis, the existence of an intra-epidermal cavity resulting from the combined effects of cytolysis and of cellular acantholysis. In this cavity there accumulate serum and sparse inflammatory cells.

In the superficial dermis microcavities appear, secondary to changes in the connective tissue fibres (demonstrable by Gomori's technique). Elsewhere, the dermis is the site of a heavy perivascular polymorphonuclear infiltrate.

With the electron microscope it can be seen that the intra-epidermal cavity results from the acantholysis of the cells of the rete mucosum which are in the process of cytolysis and of which the desmosomes are partially destroyed.

In the superficial dermis one can detect a multilocular bulla resulting from the degeneration and subsequent lysis of collagen fibres, elastic and reticulin. The basement membrane which shows little change remains adherent to the epidermis. Around this degenerate zone there is a perivascular infiltrate consisting of lymphocytes, histiocytes and, above all, of eosinophils. The capillary wall is little changed.

These dermo-epidermal changes seem to us to be secondary to the injection into the skin of enzymes contained in the saliva of insects, and are totally different from the early lesions of erythema multiforme with which they have often been compared.

Etude anatomo-pathologique des lesions papulo-pustuleuses (aphtes cutanés) au cours de syndrome du Behçet

(English translation below)

J. CIVATTE ET S. BELAICH

Les auteurs ont étudié les biopsies cutanées de lésions papulo-pustuleuses observées chez 5 malades atteints du syndrome de Behçet. Il s'agissait soit de lésions spontanées soit de lésions provoquées par la simple ponction veineuse.

Dans tous les cas où les biopsies ont été pratiquées précocément, les altérations histologiques sont comparables et paraissent différer des descriptions classiques. L'oedème dermique superficiel semble être le premier signe histologique, aboutissant ensuite à une véritable bulle sous-épidermique. Cette bulle contient essentiellement des polynucléaires neutrophiles et éosinophiles, en pourcentage variable. Parfois, l'éosinophilie est telle, qu'elle fait évoquer le diagnostic de maladie de Duhring-Brocq. Le toit du décollement est constitué par un épiderme normal non nécrosé. Le plancher conserve souvent un aspect vallonné. Au-dessous, on retrouve un infiltrat péri-vasculaire, formé avant tout de polynucléaires auxquels se mêlent lymphocytes et histiocytes. Il n'y a pas à ce stade de lésions vasculaires importantes, tout au plus un discret épaississement de la paroi des capillaires.

Sur les prélèvements biopsiques pratiqués plus tardivement, on retrouve par contre de nettes altérations vasculaires, avec dégénérescence fibrinoïde de la paroi des capillaires et infiltrat de polynucléaires souvent pycnotiques réalisant une image d'angéite allergique. A ce stade, la nécrose épidermique est franche, aboutissant à l'ulcération.

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English translation

Histopathologic picture of papulo-pustular cutaneous lesions (aphthae) in Behçet's syndrome

J. CIVATTE AND S. BELAICH

This study has been carried out in five patients with Behçet's syndrome. Skin biopsies have been performed on lesions either spontaneous or induced by venepuncture.

In all the cases where early lesions have been biopsied, the histological features are identical and appear to be different from what they are supposed to be according to classical descriptions. Superficial dermal oedema seems to be the first histological sign, turning into a true subepidermal bulla. This bulla contains neutrophils and eosinophils, the respective proportion of which varies. Sometimes, the number of eosinophils is so high as to mimic dermatitis herpetiformis. The roof of the bulla is formed of the entire epidermis which is normal, not necrotic. The base of the bulla is formed

by the top of the dermis with its papillae which stick up into the blister. Beneath, there is a perivascular infiltrate comprising chiefly polymorphonuclears with some lymphocytes and histiocytes. At this early stage, there are no vascular changes of importance, except for a possible slight thickening of the walls of the capillaries.

On the other hand, in later biopsies, there are important vascular changes with fibrinoid degeneration of the wall of the capillaries and the infiltrate often comprises pyknotic polymorphonuclears; the histological picture is that of an allergic vasculitis. At this stage epidermal necrosis is important, leading to ulceration.

Diagnosis of herpes: comparative virological studies

P.Laugier, F.Paccaud, M.Orusco, N.Hunziker and M.Monnard

Herpes is a common disease, but as well as its classical manifestations atypical forms are often seen in which diagnosis is sometimes difficult and requires laboratory assistance.

Therefore we have systematically performed virological studies of suspected herpes lesions using the following techniques:

(a) *Inoculation on cell culture* which allows the isolation and identification of the herpes virus.

(1) The material is transported to the laboratory in transport medium.

(2) Then it is inoculated on cell culture. The cytopathogenic effect is read and the herpes virus identified with herpes antiserum by seroneutralization.

(3) When the herpes virus has been isolated, the type, 1 (oral) or 2 (genital), is recognised by either immuno-electro-osmophoresis or immunofluorescence techniques.

(b) *Inoculation in vivo.* The suspected exudate is also inoculated onto the cornea of a guinea-pig, an animal as receptive as the rabbit but more economical.

In brief, this study allows us to determine the distribution of the herpes virus types 1 and 2; the value of immunofluorescence in the diagnosis of suspected herpes lesions; and the value of the inoculation onto the guinea-pig cornea for studying the two types of virus.

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Actinoreticulose survenant au decours d'une porphyrie cutanée tardive

(English translation below)

L.SCHNITZLER, B.SCHUBERT, P.BELPERRON ET J.L.VERRET

Cette observation de PCT resterait banale tant les signes cliniques biologiques et histologiques en sont typiques, si elle ne présentait la particularité évolutive, que nous ne connaissons pas jusqu'à présent, de se compliquer d'une actinoréticulose vraie.

La maladie a débuté en Juillet 1970 chez un ajusteur balancier, agé de 54 ans, par des bulles, des kystes épidermiques, une fragilité cutanée nette sur le dos des mains et le visage. Les urines sont de temps en temps fluorescentes en lumière de Wood, avec, lors des poussées, uroporphyrinurie variant entre 1,5 et 2,45 mg/l, coproporphyrinurie entre 288mg/l et 490 mg/l, porphobilinogène, néant; fer sérique entre 170 et 210 gamma/100ml, hémoglobine entre 14 et 16 g/100 ml.

L'histologie confirme l'existence d'une bulle sous épidermique caractéristique.

Les tests hépatiques sont, en outre, très perturbés chez ce sujet déprimé depuis le décès de sa femme il y a 6 ans et éthylique depuis cette date.

Après un traitement classique de méthionamine B6, Nicobion, Desféral et tentative presque réussie de sevrage, l'évolution se fait par rémission et poussées.

En 1972, se manifeste un prurit d'emblée intense avec érythème, oedème et suintement strictement localisés aux régions découvertes du visage et du dos des mains.

En Avril 1973, malgré toutes les précautions que prend depuis plusieur mois le malade pour ne pas s'exposer à la lumière et malgré les traitements locaux prescrits aussi peu allergisants que possible, une poussée de 'lucite aigüe' mène à une hospitalisation. L'obscurité absolue calme la poussée.

Au décours, nous observons la présence de nombreuses papules du dos des mains et du visage. A deux reprises le diagnostic histologique confirme notre diagnostic clinique d'actinoréticulose.

Les autres examens complémentaires pratiqués sont normaux (électrophorèse des protéines, moelle subnormale sans auto-fluorescence, absence d'anticorps antinucléaires, test au Frullania douteux).

L'étude photobiologique à l'aide du solar simulator révèle une photosensibilité extrême avec une MED inférieure à 2 secondes; une sensibilité aux UV longs tant à 2 qu'à 5 minutes.

Sur 3 tests correspondants à 5 MED, la biopsie après 3, 10 et 30 jours met en évidence le même infiltrat évocateur d'actinoréticulose.

L'examen clinique de la famille de ce patient ne permet de retrouver que la notion d'urticaire solaire chez une fille de celui-ci.

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Enfin les traitements sont très décevants à long terme, y compris les corticoïdes locaux et les vasculotropes type vitamine P à haute dose; seul semble avoir un effet photoprotecteur la crème antisolaire à la benzophénone, pour le moment bien supportée.

English translation

Actinic reticuloid developing in the course of porphyria cutanea tarda

L.SCHNITZLER, B.SCHUBERT, P.BELPERRON AND J.L.VERRET

This report of a case of porphyria cutanea tarda would be of no special interest, so typical is it from the clinical and histological point of view, if it had not run a course, of which we know of no previous example, by developing as a complication a true actinic reticuloid. A male fitter aged 54 years, first developed his illness in July 1970 with bullae, epidermoid cysts and well marked fragility of the skin on the backs of the hands and on the face. The urine has from time to time fluoresced under Wood's light and has shown during exacerbations a uroporphyrinuria ranging between 1·5 and 2·45 mg/l, coproporphyrinuria ranging between 288 mg and 490 mg/l, and no porphobilinogen. Serum iron has ranged between 170 and 210 gamma/100 ml, and haemoglobin between 14 and 16 g/100 ml.

Histological examination confirms the presence of a characteristic subepidermal bulla.

Liver function tests are very abnormal in this patient, who has been an alcoholic since the death of his wife 6 years ago.

After routine treatment with methionamine B6, Nicobion, Desferal and an almost successful attempt at abstention, the course has continued with remission and exacerbations.

In 1972 he developed pruritus which was at first intense and was accompanied by erythema, oedema and exudations strictly localised to the uncovered regions of the face and the backs of the hands.

In April 1973, in spite of the precautions which the patient had been taking for several months to avoid exposing himself to the light, and in spite of the topical treatment prescribed which was as bland as possible, a flare-up of 'acute photodermatitis' resulted in his admission to hospital. In complete darkness the attack settled. Meanwhile we had detected the presence of numerous papules on the backs of the hands and on the face. On two occasions histological examination confirmed our clinical diagnosis of actinic reticuloid. Other investigations carried out were normal (electrophoresis of the serum protein, subnormal marrow without autofluorescence, no antinuclear factor; patch test to frullania doubtful).

Photobiological investigation using a solar simulator showed extreme photosensitivity with an MED less than 2 s and a sensitivity to long ultraviolet at 2 and at 5 min.

In 3 tests with a dose corresponding to 5 MED, biopsy after 3, 10 and 30 days respectively showed the same infiltrate suggestive of actinic reticuloid.

Clinical examination of the patient's family revealed only solar urticaria in one of his daughters.

In the long term, treatment is very disappointing, including topical corticosteroids and vasculotropic drugs such as vitamine P in high dosage. Only antisolar cream containing benzophenone has seemed to have some light-screening effect and has until now been well tolerated.

Confrontations cliniques et immunopathologiques dans le lupus érythémateux

(English translation below)

J.THIVOLET

La recherche des anticorps antinucléaires sériques, des dépôts d'immunoglobulines dans la peau lésionnelle et dans la peau normale et, dans certains cas, l'étude des immunoglobulines obtenues par élution acide à partir de ces dépôts ont été réalisées chez des malades atteints de diverses variétés de lupus érythémateux discoïde chronique et disséminé aigu ainsi que chez des sujets témoins atteints de diverses dermatoses. Les résultats ont été étudiés en fonction de l'aspect clinique des lésions cutanées et des atteintes viscérales dans les lupus aigus.

Le groupe des lupus chroniques est caractérisé par l'absence d'anticorps circulants et de dépôts en peau saine, la présence de dépôts dans les lésions dans la grande majorité mais non dans la totalité des cas. Le group des lupus aigus disséminés comporte la présence constante d'anticorps circulants et de dépôts dans les lésions. Les dépôts en peau saine sont présents dans plus de la moitié des cas.

La corrélation de ces signes biologiques et des éléments cliniques fait ressortir un certain nombre d'autres éléments, en particulier, un rapport entre la présence et la persistance des dépôts cutanés à plusieurs examens successifs et la gravité évolutive notamment de la néphropathie lupique.

L'étude des groupes témoins met en évidence la fréquence relativement élevée de la présence d'anticorps anti-nucléaires dans certains groupes, notamment la pemphigoïde bulleuse et la sclérodermie. Dans la pemphigoïde bulleuse, l'association des anticorps nucléaires circulants et d'un dépôt linéaire dans les lésions ne doit pas conduire à tort au diagnostic de lupus érythémateux. Dans la sclérodermie, les anticorps antinucléaires d'un type particulier sont retrouvés également parfois fixés sur les noyaux épidermiques d'où ils peuvant etre élués. Le cas particulier du pemphigus séborrhéique de Senear–Usher est également évoqué. La signification physiopathologique de l'étude des dépôts de globuline et des anticorps élués est enfin envisagée en fonction d'expériences personnelles et des données de la littérature.

English translation

Clinical and immunopathological correlations in lupus erythematosus

J.THIVOLET

A search for serum antinuclear antibodies, deposits of immunoglobulin in lesional and normal skin and, in certain cases, the study of immunoglobulins obtained from these deposits by acid elution has been carried out in patients affected by a variety of forms of chronic discoid and acute disseminated lupus erythematosus, and also in control subjects with a variety of dermatoses. The results have been studied in relation to the clinical appearance of the lesions and to the visceral involvement in acute lupus erythematosus.

The group of cases of chronic lupus erythematosus is characterised by the absence of circulating antibody and of deposits in healthy skin, and by the presence of deposits in the lesions in the great majority but not in all the cases. The group of cases of disseminated lupus erythematosus shows the constant presence of circulating antibody and of deposits within the lesions. Deposits in healthy skin are present in more than half the cases.

The correlation of these biological signs with the clinical features brings to light a certain number of other factors, in particular the association between the presence and persistence of the cutaneous deposits over several successive examinations and the severity of the course, notably that of lupus nephropathy.

A study of the control groups reveals the relatively high frequency of the presence of antinuclear antibody in certain groups of cases, notably in bullous pemphigoid and scleroderma. In bullous pemphigoid the association of circulating antinuclear antibodies and of a linear deposit in the lesions must not be allowed to lead to an erroneous diagnosis of lupus erythematosus. In scleroderma, the antinuclear antibodies of a special type are sometimes found fixed by epidermal nuclei, from which they can be eluted. The special problem of seborrhoeic pemphigus of Senear–Usher is recalled. The physiopathological significance of the study of the deposits of globulin and the eluted antibodies is finally assessed in relation to personal experience and findings recorded in the literature.

Complément total, fractions C_3, C_4 et ponction biopsie rénale dans les connectivites dermatologiques sans atteinte rénale fonctionnelle

(English translation below)

R.TOURAINE, G.LAGRUE, J.REVUZ, MLLE HIRBEC, MME INTRATOR ET J.P.FARCET

Départements de Dermatologie, de Néphrologie, d'Anatomie-pathologie et d'Immunologie, Hôpital Henri Mondor, Paris, France

L'abaissement du taux du complément total (CH_{50}) ou des fractions C_3, C_4 au cours du lupus érythémateux systémique témoigne en règle d'une atteinte glomérulaire évolutive, d'où pour certains l'intérêt dans ces cas de la ponction biopsie rénale, même en l'absence d'anomalies dans les épreuves fonctionnelles.

Nous avons étudié le complément total et les fractions C_3, C_4 et C_3 proactivateur dans les lupus érythémateux cutanés, les sclérodermies systémiques, les morphées en plaques disséminées et les sclérodermies généralisées. Un abaissement du complément et de ses fractions, fréquemment recontré, nous a incité à pratiquer dans la majorité de ces cas, une ponction biopsie rénale. Celle-ci a révélé constamment des anomalies, soit lésions glomérulaires en microscopie optique avec dépôts d'immunoglobuline étudiés par immunofluorescence, soit seulement des dépôts en immunofluorescence.

Ces abaissements du complément, ces anomalies rénales sont des arguments supplémentaires pour l'unicité de la maladie lupique. Dans les sclérodermies, ces modifications ont été trouvées uniquement dans les morphées en plaques multiples et les sclérodermies généralisées évolutives et jamais dans les acroscléroses.

La signification et la valeur pronostique de ces anomalies complémentaires et rénales qui ne semblent pas exceptionnelles, est encore difficile à préciser. Il en est donc de même pour les indications thérapeutiques, en particulier pour l'utilisation de fortes doses de corticoïdes chez ces patients.

English translation

Total complement, fractions C_3, C_4 and renal puncture biopsy in the dermatological connective tissue disorders without functional renal involvement

R.TOURAINE, G.LAGRUE, J.REVUZ, MLLE HIRBEC, MME INTRATOR AND J.P.FARCET

The lowering of the levels of total complement (CH_{50}) or of fractions C_3 and C_4, in the course of systemic lupus erythematosus usually provides evidence of glomerular involvement, hence the interest in these cases of renal puncture biopsy even in the absence of anomalies in the function tests.

We have studied the total complement and fractions C_3 and C_4 and C_3 proactivator in cases of cutaneous lupus erythematosus, systemic scleroderma, disseminated morphoea and generalized scleroderma. A lowering of complement and its fractions which we frequently encountered led us to carry out a renal puncture biopsy in the majority of these cases. This constantly showed abnormalities, either glomerular lesions on light microscopy with deposits of immunoglobulin detectable by immunofluorescence or only the immunofluorescent deposits. This lowering of complement and these renal abnormalities provide further arguments for the unity of lupus erythematosus. Among the sclerodermas such changes have been found only in disseminated morphoea and in progressive generalised scleroderma, and never in acrosclerosis. The significance and the prognostic value of these abnormalities of complement and of the kidneys are still difficult to determine. The same applies to the therapeutic indications, in particular for the use in these patients of high doses of corticosteroids.

Swimming-pool alopecia

J.A.COTTERILL, L.MILLARD, M.M.ROBERTS, R.A.FORSTER AND J.SIKORSKI

General Infirmary, Leeds

Hair changes developed in 120 children and young adults taking part in an intensive swimming training scheme at one modern pool in Leeds. The hair of thirty-two swimmers, sixteen males and sixteen females, aged between 8 and 20 years, was examined in detail both clinically and using scanning electron microscopy. The group examined had been swimming for between 2 and 10 years and spent between 5 and 17h/ week (mean 11 h/week) in exacting training. Only ten swimmers (nine females) used bathing caps regularly.

Twenty-six subjects had significant hair changes consisting of alopecia, dry, bleached and brittle hair. Hair loss was most common in the temporal and post-auricular regions. Of the six subjects showing no hair changes, five regularly used a bathing cap; however, significant alopecia in either the temporal or post-auricular regions was seen in five other subjects who also regularly used a bathing cap.

Scanning electron microscopy showed severe cuticular damage in most hairs examined.

The clinical and microscopic appearances are thought to be due to the effect of prolonged exposure to chlorine. Greasing the hair before swimming, combined with the use of a bathing cap, has resulted in a dramatic improvement in the hair of the affected swimmers.

Mycobacterium ulcerans infection

M.S.R.Hutt

St Thomas' Hospital Medical School, London

Mycobacterium ulcerans infection, first reported in Queensland, Australia, has now been described in many areas of middle Africa, also in Mexico, Malaysia and New Guinea. The organism which grows best at 33°C is probably present in a certain type of environment and there is evidence to suggest a close association with rivers. Mycobacteria have been isolated from certain grasses in these regions and the organisms may gain access to the skin through physical contact.

The characteristic lesion is a skin ulcer with marked undermining of the edges, usually occurring on an exposed part of the body. Studies in an epidemic in Uganda have emphasized the pre-ulcerative phase of the disease, when the lesions may be small or may involve large areas of a limb. The characteristic pathology is a bland necrosis of all subcutaneous tissues, spreading eventually to the dermis and leading to ulceration. Organisms are usually present in large numbers.

Cases with long-standing lesions show a change from a necrosing to a granulomatous pattern; this is associated with the development of delayed hypersensitivity to extracts of the organism and also to tuberculin.

Chemotherapy has not proved successful in this condition, probably because a sufficient concentration of antibiotic cannot be achieved near the organisms. The standard therapeutic approach is wide excision of the necrotic areas followed by skin grafting.

The skin-gut relationship in dermatitis herpetiformis

Janet Marks, Sam Shuster, Sandra Young, R.H.Felix and A.J.Watson

University Departments of Dermatology and Pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne

Since our original concept of an indirect relationship between dermatitis herpetiformis (DH) and coeliac diseases (CD) (Shuster, Watson & Marks, 1968) the literature supporting a different relationship has 'snowballed'. The debate is hindered by the difficulties in defining DH and CD, but before new diagnostic criteria are made mandatory some general consent is necessary.

Using 'working definitions' of the two diseases we have re-examined relevant facets as listed below and as a result still adhere to our initial thesis.

(1) Natural history of DH. Spontaneous remissions occur; conversely spontaneous exacerbations necessitate significant increase in dapsone dosage above the maintenance 'base line'.

(2) IgA deposts in skin. These have not been found in all patients with DH.

(3) Incidence of CD. Over a quarter of our patients have had a small-bowel mucosal biopsy which has been structurally normal, in some cases on as many as ten occasions. Some of these biopsies have shown numbers of intra-epithelial lymphocytes within the normal range.

(4) Response of rash to gluten-free diet (GFD). Patients who have taken a strict GFD for up to 6 years and whose small-bowel has returned to normal still need dapsone. Ability to reduce or stop dapsone after a time is not confined to patients on a GFD.

(5) Genetic studies, including incidence of HL-A antigen types, suggest that there is a group of patients with DH who may not have CD.

REFERENCE

Shuster, S., Watson, A.J. & Marks, J.M. (1968) Coeliac syndrome in dermatitis herpetiformis. *Lancet* i, 1101.

Mycosis fungoides: a disease of antigen persistence

R.S.-H.Tan, C.M.Butterworth, H.McLaughlin, Samuel Malka and P.D.Samman

Westminster Hospital, London

The role of B and T lymphocytes in nineteen patients with classical Alibert's mycosis fungoides (MF) was studied. In general there was no evidence of immune depression, not even in those who had had the disease for many years. The distribution of B cells in the peripheral blood was normal. No immunoglobulin bearing lymphocytes were detected in freshly liberated cells from the skin lesions, or on direct immunofluorescence of those lesions. The mean serum IgE level was elevated even though most of the patients were not atopics. Auto-antibodies were detected in a large proportion of patients. Two cases of malignant lymphoma and one of Sézary syndrome contrasted strikingly with the MF group. Our results favour MF being a reactive granuloma to some persistent, probably exogenous, antigen. Malignancy, if it arises, is a direct consequence of this continued antigenic stimulation.

The pathology of lymphangioma circumscriptum

Dr I.W.Whimster

St Thomas' Hospital, London

A study of lymphangioma circumscriptum has been carried out for a number of years in an attempt to understand its nature and pathogenesis with a view to improving treatment (Peachey, Lim & Whimster, 1970). It will be shown that the lesion consists essentially of a collection of large muscular coated lymphatic cysterns, lying in the subcutaneous plane and communicating via dilated dermal lymphatics with the superficial vesicles.

It seems probable that these cysterns do not communicate directly, if at all, with the general lymphatic system but represent a sequestrated part of it. They have been shown by cannulation to pulsate at a slow steady rate, producing a rise and fall of pressure within them; and it is believed that the vesicles are aneurysmal dilatations of superficial lymphatics, secondary to raised pressure transmitted from the pulsating cysterns beneath. It is suggested that it may be possible to treat these lesions more successfully, and with better cosmetic results, by excising the subcutaneous cysterns and leaving the overlying skin intact.

REFERENCE

PEACHEY, R.D.G., LIM, C.C. & WHIMSTER, I.W. (1970) Lymphangioma of skin. A review of 65 cases. *British Journal of Dermatology*, 83, 519.

Reticulate pigmented anomaly of the flexures (Dowling, Degos): a new genodermatosis

E. WILSON JONES AND K. GRICE

St John's Hospital for Diseases of the Skin, London

A distinctive reticulate pigmented anomaly which initially affects the axillae and groins in young persons is described in four patients. In later life, wide areas of the skin can be affected, including the inframammary folds, the neck, and other areas on the trunk. Two of our patients were sisters, and the mother of one of the other patients was also affected, pointing to a genetic trait. The histology is characterized by the occurrence of thin branching, pigmented epidermal, strand-like proliferations arising from the lower border of the epidermis and the walls of the follicles. This histology is characteristic, and quite separate from the changes of acanthosis nigricans. We can trace only five reports of similar patients in the literature.

British Journal of Dermatology (1974) **91,** Supplement **10,** 37.

CASE REPORTS

(1) Eruptive juvenile melanomata

DR H.J. WALLACE

St Thomas' Hospital, London

M.N., female, aged 49 years. Nursing attendant. Hospital No. 31 50 05.

History. Her eruption started in 1948 on the extensor aspects of her upper arms and on her buttocks. New lesions have continued to appear over the years in the areas initially affected, and subsequently

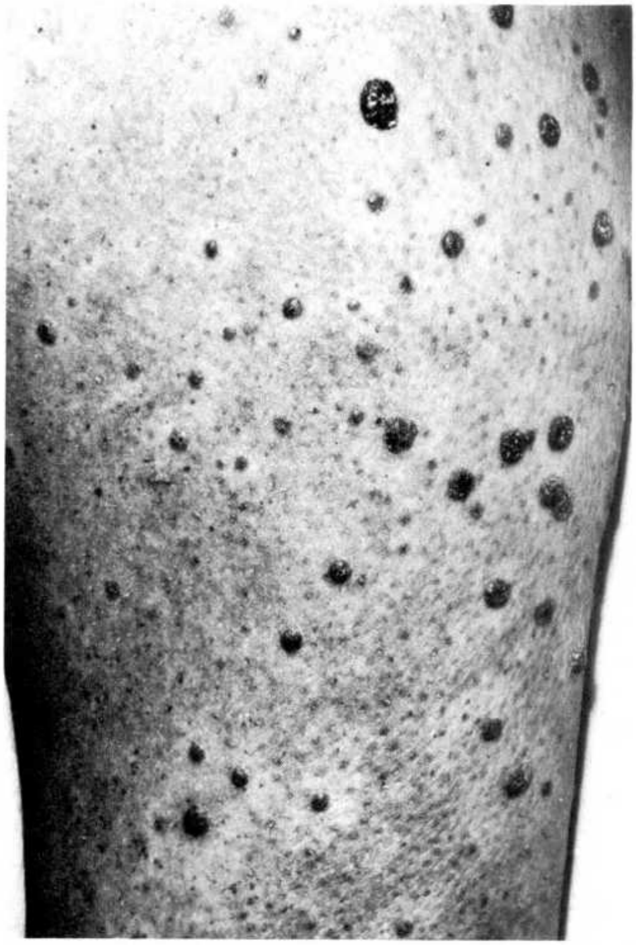


FIGURE 1. Anterior aspect of thigh showing lesions in different phases of development, some with a peripheral halo.

over most of the body, especially on the thighs and anterior abdominal wall. Palms, soles and mucous membranes have been spared. New lesions have appeared much less frequently in the past 4 years. Individual elements (Fig. 1) began as lichenoid papules, enlarging to form scaly orange coloured nodules averaging about 8 mm in diameter and 3 mm in depth, many with a pale halo. This halo disappears with heat or rubbing. Over a period of 3–4 years most lesions flatten with variable increase in pigmentation. Some become crusted and disappear. Her general health has been good.

Family history. This is unknown; she was adopted.

On examination. No other abnormal physical signs have been detected.

Investigations. Abnormal, EEG a 'diffuse non-specific abnormality without focal or epileptic features'.

Normal, blood count, ESR, urine, X-ray of chest, ECG.

Serum; immunofluorescence investigations gave negative antibody responses to the cytoplasm of fixed malignant melanoma cells, to normal skin, and to fibroblasts.

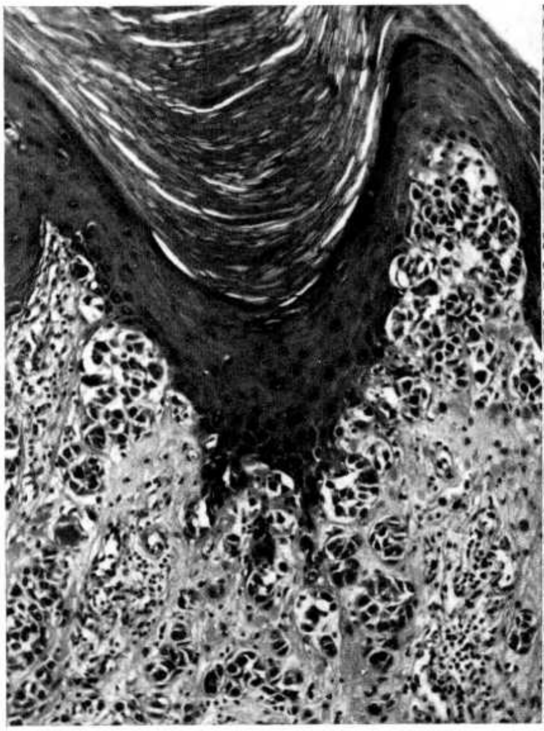


FIGURE 2. Part of the surface of a lesion showing proliferation of epithelioid melanocytes.

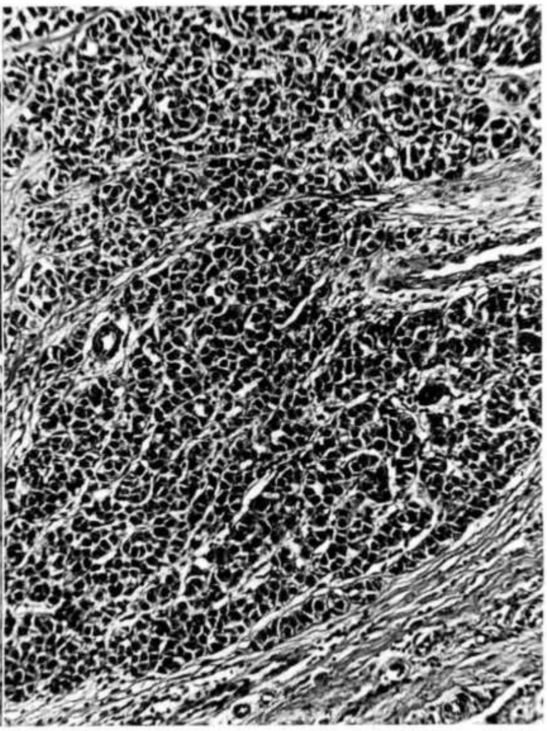


FIGURE 3. Deeper part of the same lesion.

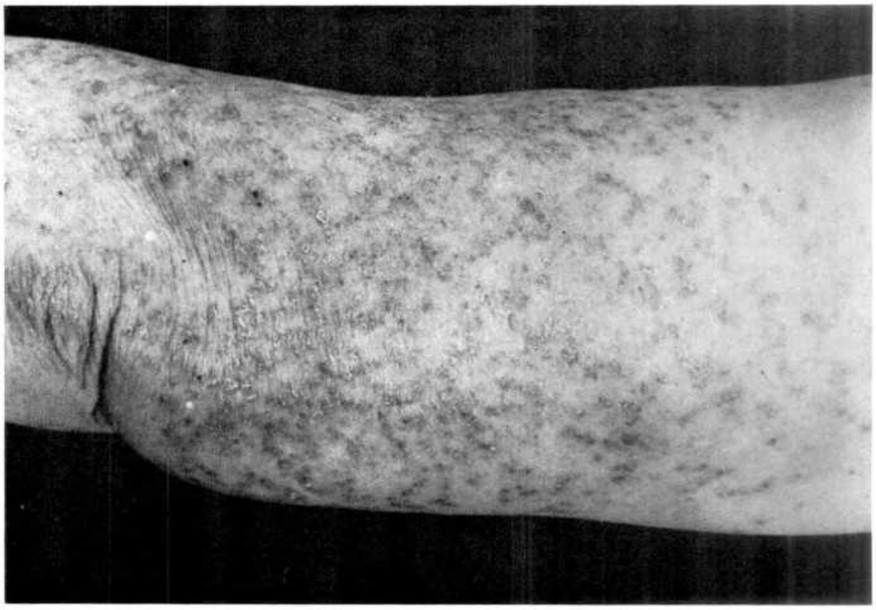
Histology. Multiple biopsies have been made. The appearances of an early lesion are indistinguishable from a malignant melanoma. Later (Figs. 2 and 3) they begin to show changes of a juvenile melanoma. The DOPA reaction is positive.

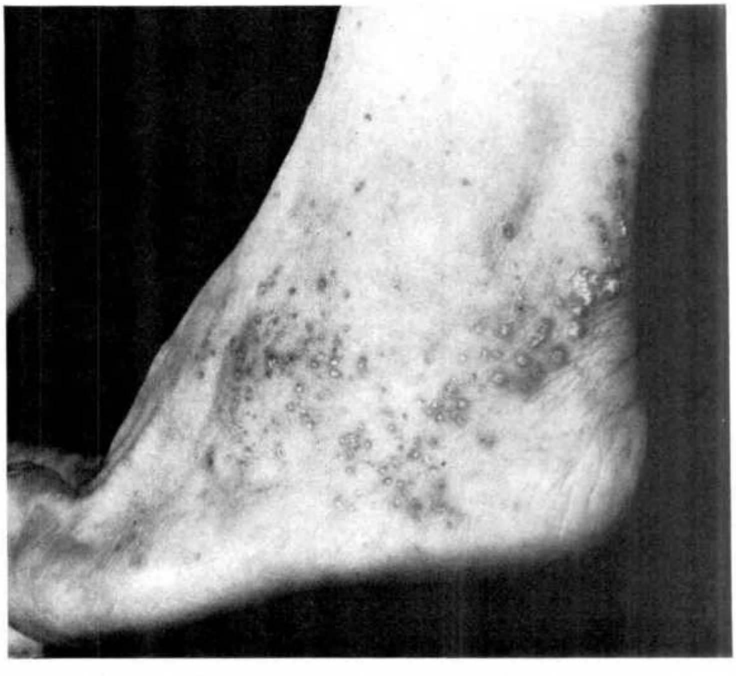
Comment. No comparable record has been found in the literature.

(2) Lichen verrucosus et reticularis of Kaposi (porokeratosis striata of Nekam)

Dr D.M.MacDonald for Dr D.I.Williams

King's College Hospital, London





FIGURES 1. AND 2. The reticulate hyperkeratotic eruption on the left arm and foot.

C.E., female, aged 55 years. Packer. Hospital case No. C63947.

History. In 1956 she developed a scaly rash on her right arm, subsequently spreading to the left arm and lower legs. In 1958 she developed a scaly rash on her face, and since then the eruption has progressed with some fluctuation (Figs. 1 and 2).

On examination. There is an erythematous squamous rash on the face, involving the eyebrows, forehead and nose. On the arms and legs there is a reticulate hyperkeratotic eruption.

Investigations. These have all been negative including ANF; LE cells; WR; serum proteins; ESR; full blood count; chest X-ray; renal and liver function tests.

Histology. Hyperkeratosis and considerable parakeratosis with follicular plugging are present. There is widespread basal liquefaction and numerous Civatte bodies. The lymphohistiocytic crater in the dermis extends into the epidermis, which itself is atrophic in parts. Direct immunofluorescence staining shows many cytoid bodies of IgM in the dermis. Fibrin is also deposited in the papillary dermis and sharply outlines the epidermo-dermal junction and also many of the cytoid bodies. These changes, although not diagnostic, are characteristically seen in lichen planus. There are no features of LE.

Course and treatment. Over the years treatment has included salicylic acid ointment, vitamin A acid ointment, topical steroids with occlusion, vitamin A (orally), dapsone and chloroquine. There has been a doubtful response to chloroquine on two occasions.

Comment. While this clinical pattern conforms to the above diagnosis, certain features of this condition suggest a relationship with lupus erythematous.

(3) Primary biliary cirrhosis presenting with pigmentation and prurigo

DR E. LIPMAN COHEN AND DR J. R. BILLINGHURST

Oldchurch Hospital, Romford, Essex

I.G., female, aged 48 years. Hospital No. 138600.

History. For 4 years she has had gradually increasing skin pigmentation resembling a sun tan. For 2 years there has been undue itchiness of her skin with occasional itchy lumps in the skin. For 1 year she has had transient episodes of mild obstructive jaundice lasting about 2 weeks. Her general health remains excellent. She takes quinine for cramps in the legs.

On examination. There is moderate generalized skin pigmentation, resembling sun tan, with sparing of skin scars, skin creases and mucous membranes, and most marked on the lower part of her back and on her neck. There are a few hard prurigo papules, some slightly excoriated.

Investigations (relevant). Alkaline phosphatase: 18, 30, 63.2, 57.9 units/100 ml. Serum albumin: 3.0, 2.7 g/100 ml. Serum globulin: 4.4, 4.5 g/100 ml. IgG 1.25%; IgM 240 mg%; IgA 250 mg%. Polyclonal increase in gamma globulin. Mitochondrial fluorescent antibody positive, titre 1:160. Normal serum iron, blood glucose, plasma cortisol and electrolytes. Normal cholecystogram.

Histology (St John's Hospital). There is a mild lymphohistiocytic infiltration around the upper dermal vessels, with marked pigmentary incontinence.

Comment. The positive mitochondrial antibody test, normal alkaline phosphatase and high IgM establish the diagnosis of primary biliary cirrhosis. The presentation with prurigo and pigmentation several years before any jaundice is very interesting. So far jaundice has only been transient.

(4) Scleroderma with primary biliary cirrhosis

DR R. CLAYTON FOR DR R. J. CAIRNS AND DR M. FEIWEL

St Bartholomew's Hospital, Kent and St Mary's Hospital, London

E.D.P., female, aged 53 years. Training course organizer.

History. For 2 years the patient has been 'dimpled all over' with tightness of the skin of the forearms and lower legs (Fig. 1). There is no history of Raynaud's phenomenon.

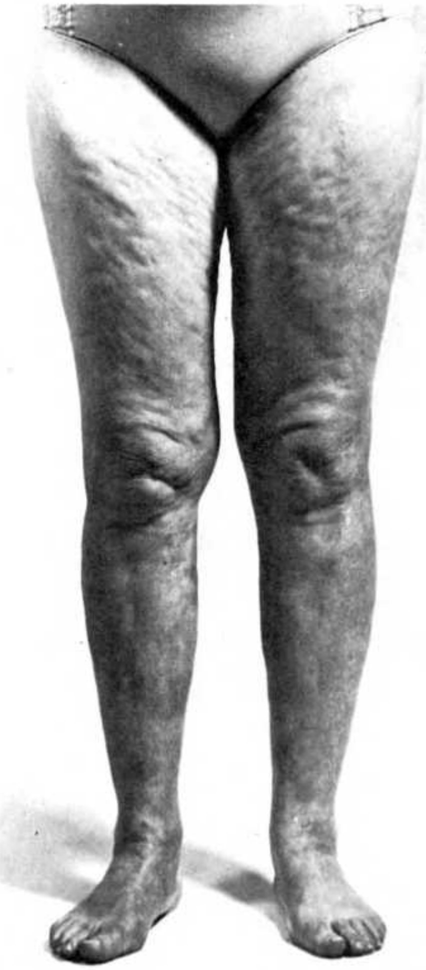


FIGURE 1. Dimpling of skin overlying areas of thick sub-cutaneous fat.

In 1971, a left mastectomy for intraduct carcinoma was performed.

On examination. The skin overlying areas of thick sub-cutaneous fat, especially of the abdomen and thighs, shows dimpling. There are sclerosis and smoothness of the skin of the forearms and lower legs, especially the ankles. There is no telangiectasia, calcinosis or acrosclerosis.

Investigation. Blood count; ESR; blood urea; WR; liver function tests, serum proteins and immunoelectrophoresis; urine; ECG; chest and hand X-rays; barium swallow, meal and follow through; no abnormality detected.

LE cells; Rheumatoid factor; ANF, were negative.

Mitochondrial and smooth muscle antibodies were positive.

Biopsies. Thigh skin shows some thinning of the main body of the dermis with superficial sweat glands. The lower part of the dermis shows a sparse lympho-histiocytic infiltrate with some irregularity of elastic tissue and some collagen bundle hyalinization compatible with atrophic scleroderma (E. Wilson Jones).

Liver shows non-specific inflammatory changes with a non caseating portal zone granuloma compatible with early primary biliary cirrhosis.

Comment. Primary biliary cirrhosis has recently been reported to occur with the spectrum of sclerodermatous disease, generalized scleroderma, systemic sclerosis and the CRST syndrome (calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia of the Osler-Rendu-Weber type, with frequent disturbance of oesophageal motility). Six out of forty-one cases of primary biliary cirrhosis have been reported to be associated with the CRST syndrome. Serum IgM is frequently raised and the addition of keratoconjunctivitis sicca (Sjögren's syndrome) has been described. It has been suggested that the association of primary biliary cirrhosis and the sclerodermatous process is probably more than coincidental and that the two diseases may have a common auto-immune basis. The appearance of the skin in this case is unusual.

(5) Widespread atypical lichen amyloidosus, primary biliary cirrhosis and scleroderma

DR MARTIN M.BLACK

St John's Hospital for Diseases of the Skin, London

Q.J.R., female, aged 45 years.

History. In 1969 she first developed a pruritic papular eruption in both cubital fossae. Since then the condition has progressively spread to involve the lower forearms and dorsa of hands and fingers. A similar eruption soon appeared in the popliteal fossae and has spread to involve the thighs, shins, dorsa and soles of feet. Since 1972 the pruritus has become more severe and she has noticed increasing pigmentation of the trunk, head and neck. Her general health remained good until 1972 but since then she has complained of malaise and developed mild Raynaud's phenomenon of the fingers. Her history also included herpes zoster in November 1973.

Family history. Nil significant.

On examination (June 1970) there were revealed numerous small, flat-topped shiny papules (Fig. 1) in both cubital fossae. These were also present on the flexor aspect of the lower forearms and extensor aspect of the lower legs. Several papules were arranged in a linear distribution suggestive of a Köbner phenomenon and a series of scratches, sufficient to produce minute bleeding points, induced further papules in the line of the scratches after an interval of three weeks.

March, 1974. In addition to the above sites, the papular eruption has spread to involve the thighs, dorsa of the feet (Fig. 2), hands and fingers (Fig. 3) and also the soles. She displays a diffuse macular hyperpigmentation of the trunk, limbs, head and neck, but this is most pronounced over the lower

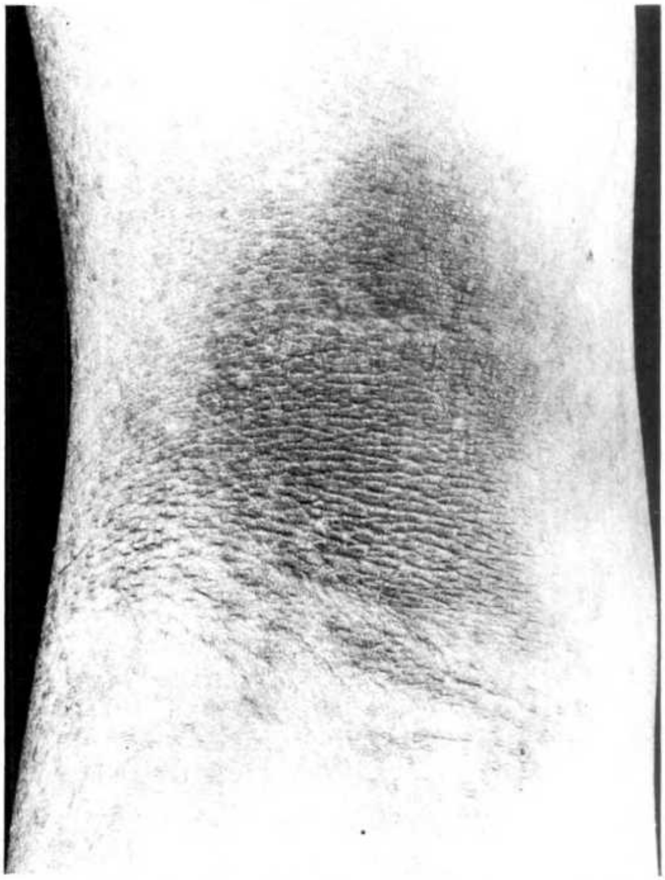


FIGURE 1. Cubital fossa: clusters of shiny flat-topped pigmented papules.

back and inguinal region. The skin over the dorsa of the hands and extensor aspect of the forearms appears tethered, and the nail fold capillaries are tortuous. She is not jaundiced; the liver is moderately enlarged but the spleen is impalpable.

Investigations. June 1970. Hb 94%, WBC 7000 mm^3 (normal differential). ESR 22 mm/1 h; Serum proteins: 8·1 g/100 ml, electrophoresis revealing a slight increase in $\alpha 2$ globulin; ANF negative; Urine: no albuminuria or Bence Jones protein could be detected. Histopathology: cubital fossae and lower leg showed hyperkeratosis and acanthosis with a few hyaline bodies in the lower epidermis; small hyaline deposits in the papillary dermis which stained positively for amyloid.

November 1973. Hb 13.8 g, ESR 75 mm/1 h. Liver function tests: serum bilirubin: 1·0 mg/100 ml; serum alkaline phosphatase: 85·0 KA units/100 ml, thymol flocculation + + +; serum proteins: 8.8 g/100 ml, electrophoresis revealing a slight increase in $\alpha 2$ and a general increase in gamma globulin. ANF and smooth muscle antibody: negative; mitochondrial antibody: positive to greater than 1 in 40; Immunoglobulins: IgG 2340 mg/100 ml, IgA 405 mg/100 ml, IgM 560 mg/100 ml. Barium swallow: no evidence of oesophageal varices. Schirma test for sicca syndrome: negative.

Liver biopsy: extensive fibrosis and some nodule formation. Bile duct proliferation and moderate

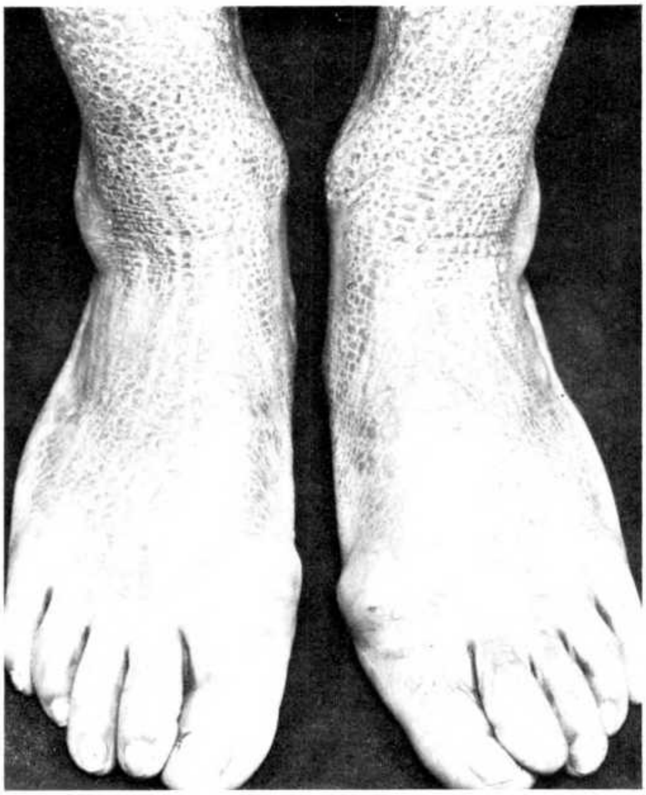


FIGURE 2. Extensive papular lichen amyloidosus on ankles, dorsa of feet and toes.

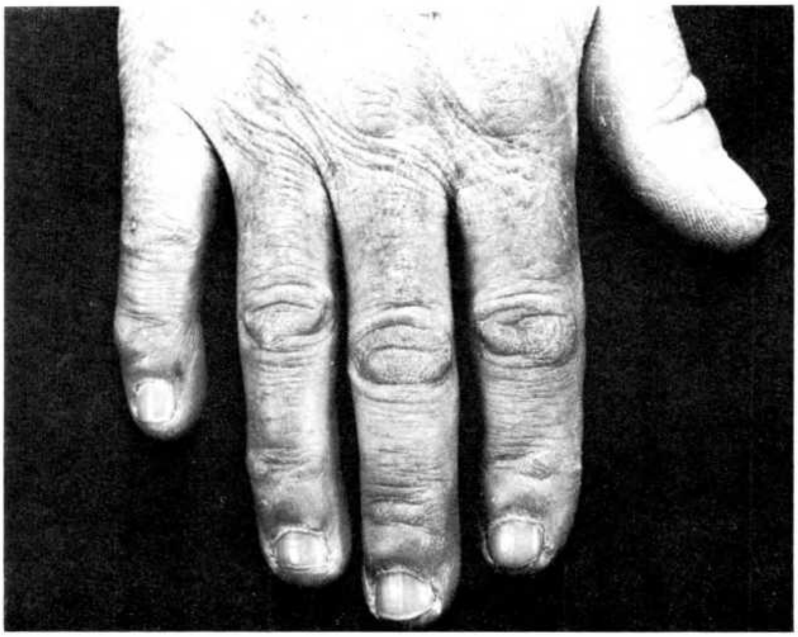


FIGURE 3. Hyperkeratotic thickened plaques on dorsa of hand and fingers.

acute and chronic inflammatory infiltrate. No cholestasis, granuloma formation or deposition of amyloid. The appearances favour primary biliary cirrhosis.

Rectal biopsy: no deposition of amyloid. Biopsy of tethered skin of forearm: sclerosis of dermal collagen suggestive of scleroderma. Focal deposition of amyloid in the papillary dermis.

Treatment. Since November 1973 has been with cholestyramine 4 g daily and topical corticosteroids to papular lichen amyloidosus has produced little relief so far.

Comment. Papular lichen amyloidosus most commonly affects the extensor surface of the lower limbs. The prominent involvement of the flexures, dorsa of hands and feet and even the soles appears to be most unusual. This patient is believed to be the first recorded association of lichen amyloidosus and the development of the Köbner phenomenon (Black, 1972a). Three cases have been described who have developed warty lichen amyloidosus on the dorsa of the fingers in association with long-standing systemic sclerosis (Black, 1972b). The present case has developed a similar warty thickening on the dorsa of the hands and fingers in association with extensive lichen amyloidosus elsewhere. More recently she has developed Raynaud's phenomenon and has scleroderma of the forearms and hands. The association of primary biliary cirrhosis with systemic sclerosis has been appreciated for some time (Murray-Lyon *et al.*, 1970; Reynolds *et al.*, 1971) suggesting that these two diseases might have a common autoimmune basis. The majority of these cases have had features of the CRST syndrome (calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia) but two patients have been described with scleroderma alone (Morgan, 1973). This patient also does not have features of the CRST syndrome. The presence of widespread lichen amyloidosus, primary biliary cirrhosis and scleroderma appears to be unique, but may be no more than just an extraordinary coincidence.

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(6) Ulcerative colitis, systemic sclerosis and necrobiosis

DR H.J.WALLACE

St Thomas' Hospital, London

D.H., female, aged 68 years. Housewife. Hospital case number 51 89 98.

History. In 1969 she developed Raynaud's phenomenon in her hands. In 1973 painless transient flesh coloured nodules appeared on her forearm and thighs, and violaceous plaques on her legs.

Thirty years ago mastitis was treated by mastectomy. Since 1957 she has had recurrent ulcerative colitis, usually well controlled with codeine.

On examination. She is plethoric. There is scleroderma of her hands, and to a lesser degree of her face and limbs. On her shins there are superficial plaques, violaceous at the periphery. Blood pressure 130/80.

Investigations. Blood. Abnormal ESR 30 mm/1 h; ANF positive 1/1250. Normal or negative: Hb, WBC, film, Rose Waaler, Latex, mitochondrial antibodies, electrolytes, urea, creatinine, calcium, phosphorus, serum proteins and electrophoresis, bilirubin, alkaline phosphatase, SGOT.

Urine. Normal, no glycosuria 1 h after 50 g of glucose orally. X-ray, chest normal. Supine barium swallow, impaired peristalsis consistent with systemic sclerosis. Barium enema, loss of haustral pattern throughout the colon from the hepatic flexure downwards and slight irregularity of the bowel wall in the sigmoid colon.

ECG normal. Sigmoidoscopy, typical ulcerative colitis, with occasional pseudopolyps.

Biopsy. Skin. Right forearm, necrobiotic foci in dermis; leg, diffuse necrobiosis with occasional small foci of sarcoid like granulomata.

Rectum. Florid non-specific proctitis; no granulomata seen; appearances compatible with ulcerative colitis.

Comment. The association of ulcerative colitis and systemic sclerosis is not common but has been reported from time to time (Bicks, Goldgraber & Kirsner, 1958; DeLuca, Spiro & Thayer, 1965; Lushbaugh, Rubin & Rothman, 1948; Sackner, 1966).

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(7) Blood hypercoagulability causing leg ulceration

DR R.S.-H. TAN AND DR P.W.M.COPEMAN

Westminster Hospital, London

A.I., male, aged 38. Businessman.

History. He has had painful ulcers on his lower legs for 16 years with a sudden recent extension of ulceration into a surrounding livedo reticularis pattern on his feet and ankles. A skin graft (1969) broke down 2 days after mobilization.

For 5 years he has also had a feeling of intense hunger if without food for more than 2–3 hours.

Past history. He had had recurrent thrombophlebitis for 3 years before the onset of ulcers.

Family history. He originally came from Eastern Europe. His grandfather had his leg amputated for gangrene.

On examination. There are large necrotic ulcers on the lower legs and feet (Fig. 1) with surrounding scars. There is also a reticulate pattern of ulcers and hyperpigmentation. His feet are warm and peripheral pulses normal. General examination: otherwise healthy. No signs of Behçet's disease.

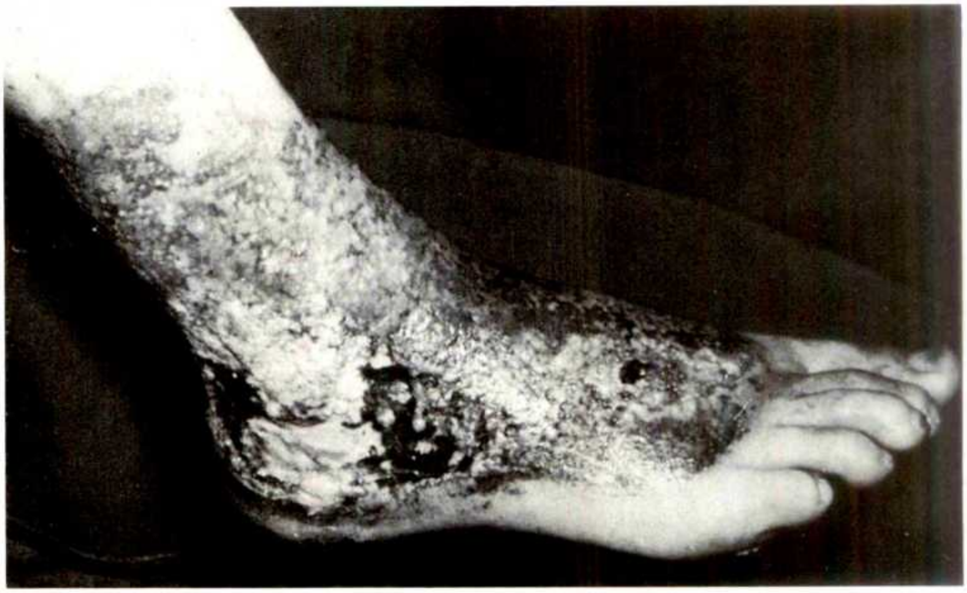

FIGURE 1. Necrotic ulcer of the lower leg and foot.

Investigations. Clot weight fibrinogen: 1800 mg/100 ml (November 1973), 260 mg/100 ml (January 1974), normal 200–400 mg/100 ml. Euglobulin lysis time $4\frac{1}{4}$ h (November 1973), $2\frac{1}{4}$ h (January 1974), normal $1\frac{1}{2}$–4 h. Platelet aggregation time with ADP: 21 s (November 1973), 55 s (January 1974), normal 24–45 s. Complement studies: activated C3—2.8 T units (1 mg $\approx$ 2 T units). Protein electrophoresis: increased α_2 globulin.

Glucose tolerance test (1969, 1973): flat, 70–89 mg/100 ml. Fasting blood sugars (1969, 1973): 46–81 mg/100 ml. Carcino-embryonic antigen: 33 ng/100 ml (November 1973) (control 10 ng/100 ml), 40 ng/100 ml (January 1974).

Negative or normal results. Thrombin time, prothrombin time, kaolin–cephalin clotting time, fibrinogen titre, fibrin degradation products, immunoelectrophoresis with antifibrinogen, platelet count, cryoglobulins, cryofibrinogen, cold agglutinins, immunoglobulins, Hb electrophoresis, RBC osmotic fragility, sickling test, Wasserman reaction, fluorescent treponemal antibody, autoantibody screen, LE cells.

24-h fasting blood sugar, xylose absorbtion, faecal fats, liver function tests, Hb, WBC, ESR, urea electrolytes. X-rays: chest, feet and ankles.

Histopathology of ulcer. Chronic inflammation, endarteritis of small vessels, some with organizing thrombus.

Treatment. Vertical elevation drainage of the legs for 20 min three times a day, since end of October 1973. Phenformin 50 mg twice daily and ethyloestrenol 4 mg twice daily since 27 November 1973.

Course. Ulcers healed in 3 months.

Comment. Abnormalities of blood coagulation may be important in the pathogenesis of the leg ulcers due to intravascular sludging, hyperviscosity, coagulation and decreased intravascular fibrinolysis (Copeman & Ryan, 1971). Our patient had a grossly elevated level of normal fibrinogen in the plasma. Dormandy *et al.* (1973) have recently emphasized the critical role of plasma fibrinogen in determining the blood viscosity. Smith & Holder (1971) have reported on the occurrence of stasis ulcers in one member of a family with an inherited disorder of abnormal (Baltimore) fibrinogen and a tendency to venous thrombosis.

Since he started fibrinolytic treatment, the ulcers have healed rapidly, and the coagulation tests have also returned to normal.

Elevated fibrinogen levels (Brugarolas *et al.*, 1973) and superficial thrombophlebitis are associated with internal cancer; hypoglycaemia may be associated with pancreatic islet cell tumours or non-pancreatic tumours such as retroperitoneal fibromas or sarcomas (Lowbeer, 1961). At present, he has no clinical evidence of these conditions. However, we are anxious about the increasing levels of carcino-embryonic antigen (*British Medical Journal*, 1972) which may be ominous, especially as the leg ulcers are healing.

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(8) Malignant lymphoma of the skin treated with bleomycin

Dr Michael R. Klaber and Dr Dowling D. Munro

Department of Dermatology, St Bartholomew's Hospital, London, E.C.1

M.D., male, aged 79 years. Retired photo-engraver.

History. In 1971 he developed recurrent epistaxes and underwent full haematological investigation. In June 1973 he noticed a firm erythematous lump on his forehead which gradually enlarged and by October 1973 similar lesions had begun to appear on his eyelids, cheeks and the rest of his body. They would begin as red rings and become indurated.

On examination. In the centre of his forehead there was a large indurated erythematous plaque about 7 cm across (Fig. 1). There were similar lesions on his left eyelid, both cheeks, and profusely scattered over his back (Fig. 2), chest, arms and thighs. There was no hepatosplenomegaly and no lymphadenopathy. General examination was unremarkable.

Investigations. Hb 11.3 g/100 ml, MCV 111 fl., WBC 5300/mm^3 with 39% neutrophils, some of which had atypical features, and 17% atypical mononuclear cells. Reticulocytes 1.2% and ESR 60 mm in 1 h. Serum iron 50 μg/100 ml, iron binding capacity 270 μg/100 ml. Blood urea 55 mg/100 ml.

Antinuclear factor positive at a titre of 1/320. Immuno-electrophoresis: IgG 1230 mg/100 ml (normal 700–1800), IgA 465 mg/100 ml (normal 80–400), IgM 305 mg/100 ml (normal 40–250). Bone marrow biopsy favoured a diagnosis of 'preleukaemia'; there was no evidence of infiltration by cells derived from a malignant lymphoma. X-rays of chest, abdomen, post-nasal space and sinuses—normal. No Bence-Jones proteinuria. Skin biopsy—forehead and right upper arm: 'fairly diffuse infiltration of corium with atypical lymphocytes, many of which are of intermediate type with frequent mitotic figures (Fig. 3). This is a malignant lymphoma.'

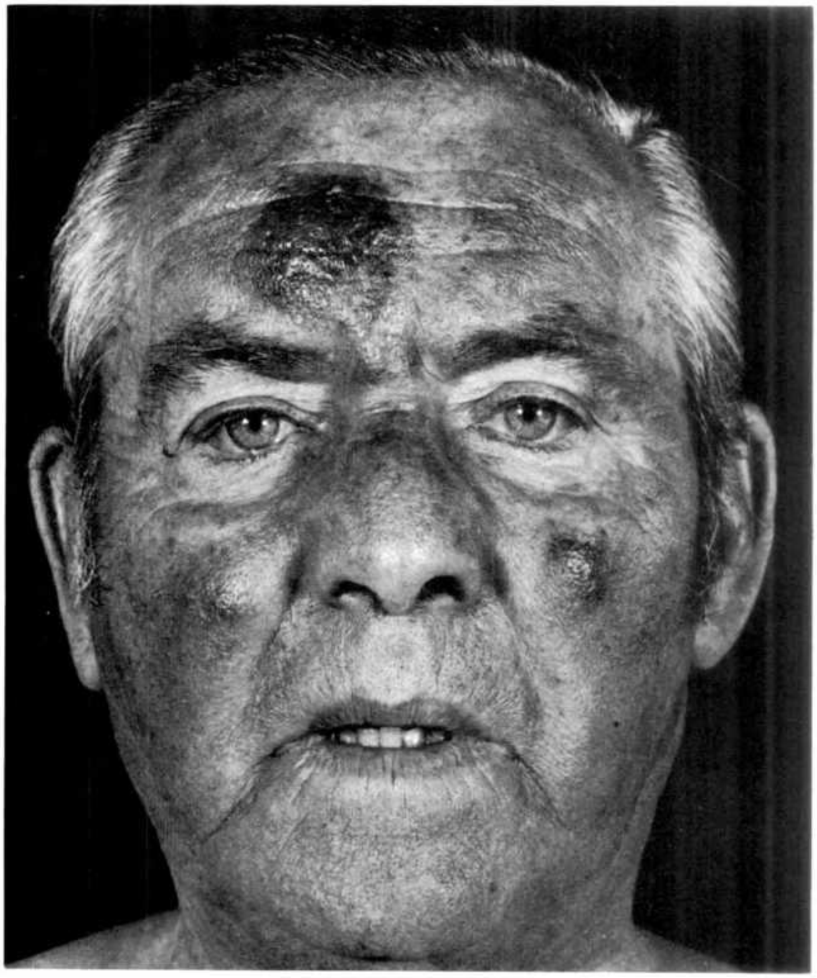

FIGURE 1. Lymphomatous deposits on forehead, cheeks and left eyelid.

Course and treatment. He was initially treated with radiotherapy to the face (1000 rad at 140 kV). The treated lesions all became less infiltrated and diminished in size. Over the next 3 weeks he deteriorated very considerably, developing hepatomegaly, and the possibility of electron beam therapy was considered. However, he was readmitted and received a further 1000 rad at 140 kV following which his skin lesions temporarily regressed, but because he developed new lesions he was started on prednisolone 40 mg daily. The liver was by now impalpable but a repeat bone marrow biopsy showed

20% blast cells. In February 1974, further cutaneous lymphomatous lesions appeared on his face, back and arms and he was started on bleomycin 1 mg sub-cutaneously daily by self-injection. The nodules on his face then resolved and his other lesions became flatter and ill-defined.

Comment. This man's malignant lymphoma cutis had relapsed following two courses of radiotherapy and steroid therapy. He has shown an excellent response to bleomycin 1 mg sub-cutaneously daily. Bleomycin is the name for a group of antineoplastic antibiotics isolated from a strain of *Streptomyces verticillus* found in a Japanese coal mine (Umezawa, 1965). It inhibits cell division in the early stages of mitosis, probably mainly by causing scission of single stranded DNA. It appears to be concentrated almost exclusively in the skin and in the lungs (Ishizuka *et al.*, 1967), with little or no myelotoxicity. It would, therefore, seem to be an ideal drug for treating cutaneous malignancy. As might be expected, toxic effects have mainly involved the lungs, where after total doses in excess of 300 mg there is a small but definite incidence of pneumonitis and pulmonary fibrosis, leading to death in from 2 to 8% of cases (Yagoda *et al.*, 1972). Mucocutaneous side effects include changes frequently seen with other chemotherapeutic agents, such as alopecia, stomatitis and nail changes, as

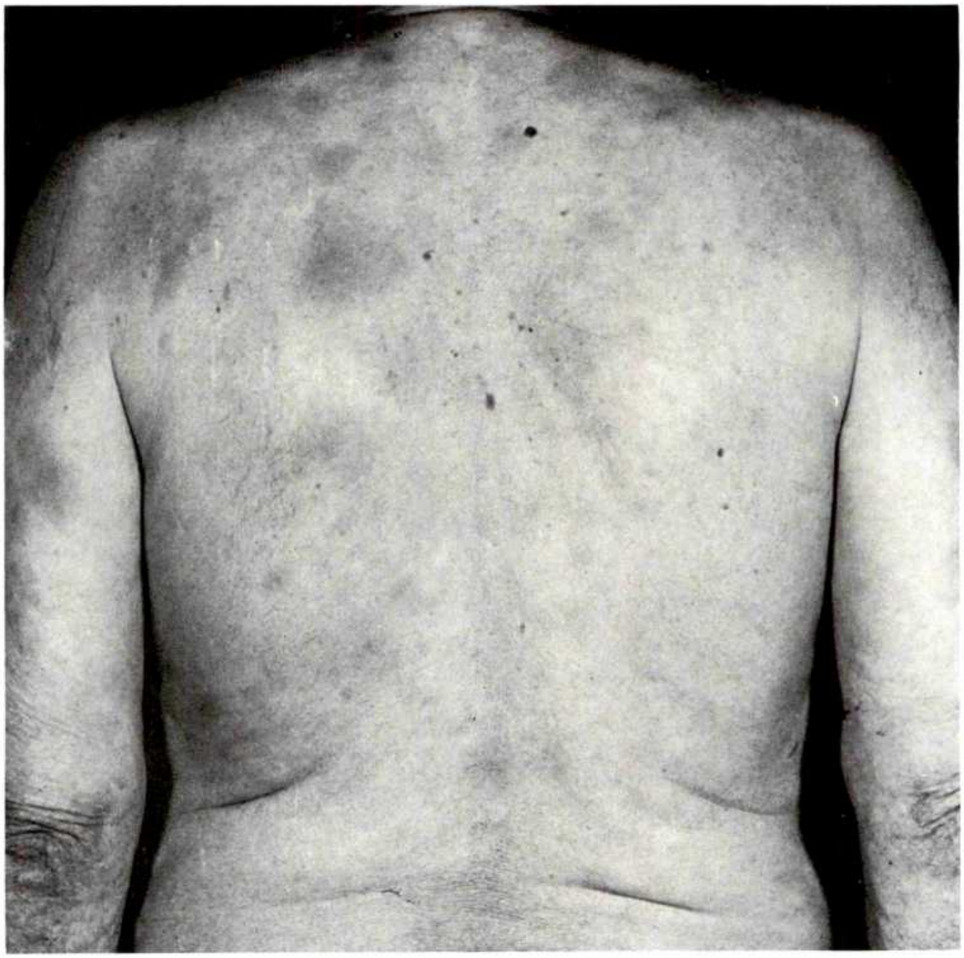

FIGURE 2. Showing distribution of lesions on back.

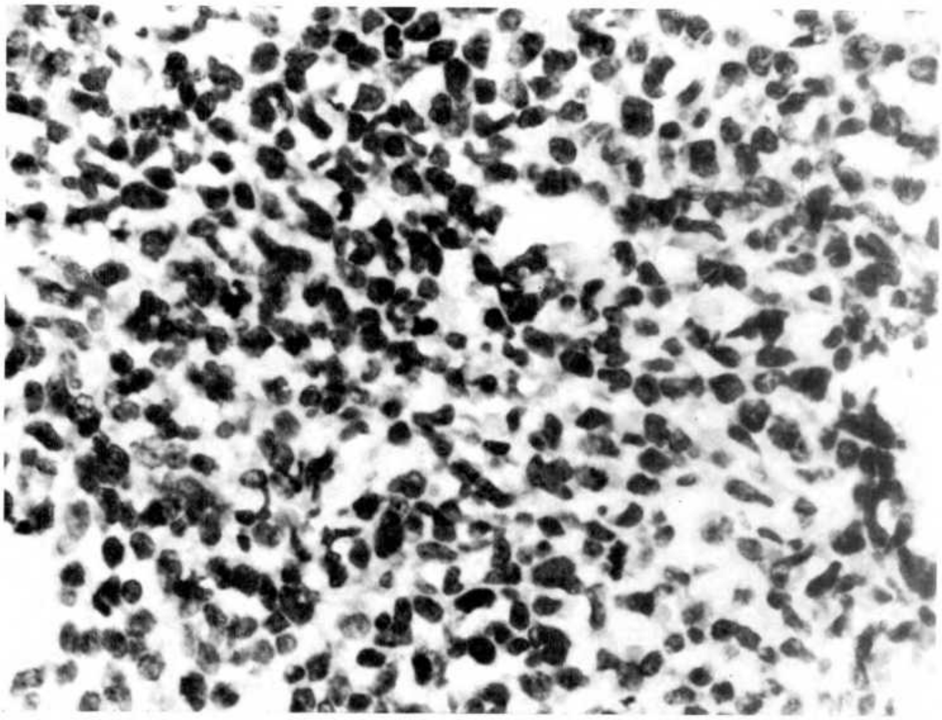

FIGURE 3. Histopathology showing diffuse infiltration of corium with atypical lymphocytes having frequent mitotic figures (× 600).

well as more unusual effects—linear hyperpigmentation, infiltrated plaques on the hands and a scleroderma-like process (Cohen *et al.*, 1973).

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(9) Mycosis fungoides treated with bleomycin

Dr Michael R. Klaber and Dr Dowling D. Munro

Department of Dermatology, St Bartholomew's Hospital, London

J.C., male, aged 63 years. Catering manager.

History. Seven years ago he developed pink, scaly, slightly itchy patches on the left forearm; these progressively spread, became redder and more itchy. Skin biopsy 4 years ago supported the clinical diagnosis of a premycotic eruption; a lymph-node showed only dermatopathic lymphadenitis. He was treated with systemic steroids without benefit and, as he deteriorated, chlorambucil and then radiotherapy (1500 rad at 70 kV) with some temporary benefit. An identical twin has had alopecia areata but no other skin disease.

On examination (December 1973). He showed numerous well defined, infiltrated erythematous plaques scattered over his whole body (Fig. 1), general thinning of body hair with patchy loss in beard and eyebrows, and palmar hyperkeratosis. There was no lymphadenopathy or hepatosplenomegaly.

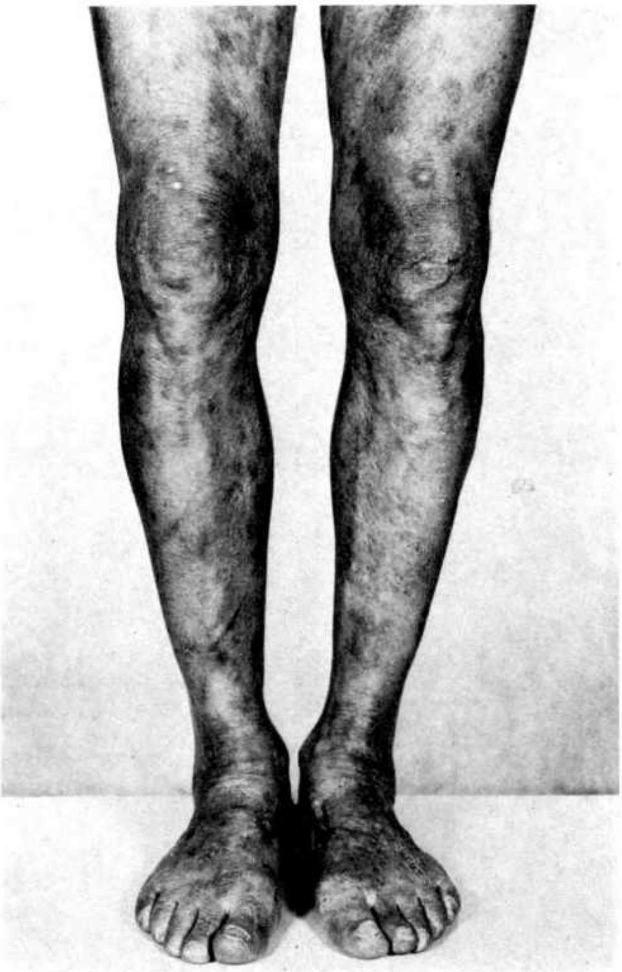

FIGURE 1. Showing distribution of lesions on legs. Only the hyperpigmentation remains.

Investigations. Hb 15.1 g/100 ml, WBC 6500/mm^3 with 70% polymorphs, ESR 10 mm in 1 h. Platelets normal. Blood urea, electrolytes, liver function tests, serum calcium and phosphate, serum proteins, urine, X-rays of chest and lumbar spine were all within normal limits. X-rays of pelvis showed ill defined lytic areas in the right femoral head. Histopathology of skin from scalp and left elbow flexure showed some dermal scarring with a moderately dense lympho-histiocytic infiltrate containing several hyperchromatic cells.

Course and treatment. Electron beam therapy or topical nitrogen mustard therapy were both considered but he was treated instead with bleomycin 1 mg daily sub-cutaneously. He responded very well; the lesions cleared though occasional new ones appeared and lasted for a week or two. There was some hyperpigmentation at the sites of previous lesions (as shown in Fig. 1), which is a feature of bleomycin therapy.

Comment. This patient's mycosis fungoides with generalized infiltrated plaques responded well to bleomycin therapy. Spigel & Coltman (1973) reported two cases of mycosis fungoides refractory to all other modes of therapy, who went into remission with bleomycin. They recommended that maintenance of remission should be with a combination of bleomycin and cyclical prednisone and cyclophosphamide. Blum, Carter & Agre (1973) reviewed data from 1174 patients treated with bleomycin and concluded that its use as a single antineoplastic agent should be in end stage patients resistant to conventional therapy or disabled by side effects, especially myelotoxicity. They found it to be uniformly active against all types of lymphoma, with a 5% complete response rate and a 33% partial response rate for the 198 patients evaluated.

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(10) Relapsing polychondritis

PROFESSOR C.D.CALNAN

St John's Hospital for Diseases of the Skin, London

P.M., male, aged 55 years, Surveyor.

History. Nine months ago this man noticed irritation behind his ears from his spectacle frames. The ears then became painful and swollen and he noticed some degree of deafness.

Two months ago he complained of 'catarrh' with a hoarse voice and 1 month ago he had painful knees and elbows. He also noticed some soreness of his nose which made breathing through it difficult.

In the past he has had infective hepatitis, malaria and eczema.

Family History. One daughter has eczema.

On examination. Both ears show irregular indurated swelling and redness but the lobes of the ears are noticeably spared.

Investigations. ESR 85 mm in 1 h; Hb 12·7 g/100 ml; white cell count 8300/mm^3. Rose Waaler test negative; ANF negative. Immunoglobulins; IgG 2000, IgA 360, IgM 90 mg%. Albumin 3·4%, globulin 4·1%. Histology, typical changes of relapsing polychrondritis.

(11) Familial acne conglobata, hidradenitis suppurativa, pili torti and cataracts*

DR S.C.GOLD AND DR T.J.DELANEY

St George's Hospital, London

D.H., male, aged 35 years.

History. He developed acne conglobata together with axillary and groin hidradenitis when aged 14. These lesions have continued to trouble him despite prolonged treatment with systemic antibiotics and repeated minor surgery. Recently, a large area of his neck skin was removed and replaced by a split skin graft with marked improvement.

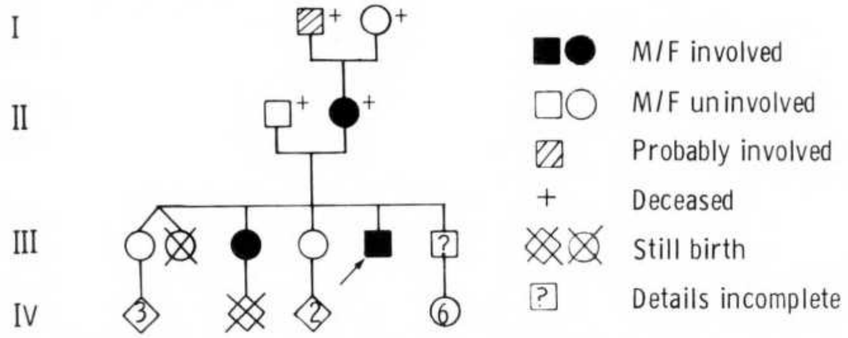

FIGURE 1. Familial acne conglobata, hidradenitis suppurativa, pili torti and cataracts—family tree.

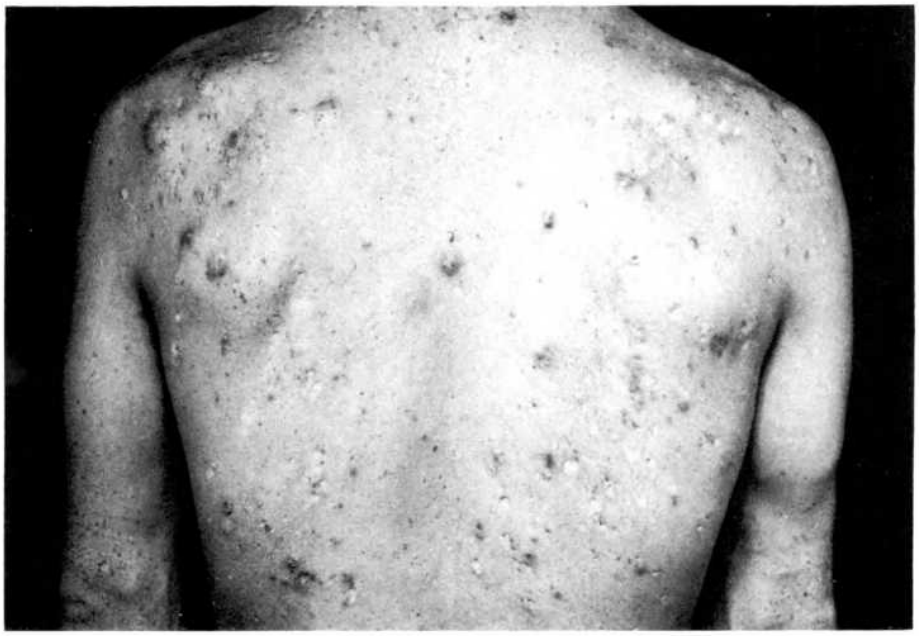

FIGURE 2. Back showing extensive acne scars and cysts.

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* Previously shown at the meeting of the Section of Dermatology of the Royal Society of Medicine, 21 October 1971.

He was known to have poor vision in childhood, due to cataracts. The cataract from the right eye was removed in 1970. It showed no unusual features but appeared to have abnormal attachments.

His hair has always been short and sparse.

Family History (Fig. 1). One sister is similarly affected; neither he nor she have live children. His mother is known to have had the same conditions, eventually dying of a squamous carcinoma arising in a chronic skin sinus. His grandfather is believed to have had similar skin, hair and cataracts.

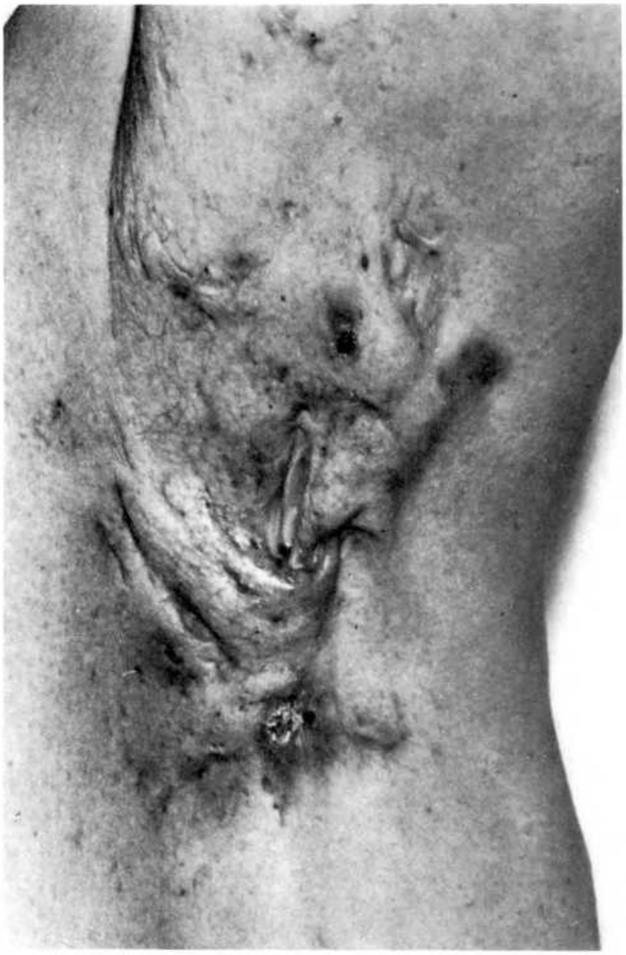

FIGURE 3. Axilla showing sinuses and scarring.

On examination there is extensive acne conglobata of neck and chest with hidradenitis suppurativa of axillae, perineum and groins (Figs. 2 and 3). Acneiform lesions are present in the antecubital fossae. His scalp shows sparse twisted hairs (Fig. 4).

Investigations. Scanning electron microscopy (Dr R. Dawber) showed hair shafts of varying diameter and cross section with characteristic twists of differing degree. The scale pattern was normal (Fig. 5). Urinary amino acids, routine blood count and chromosome analysis were normal.

Discussion. The transmission of these conditions in this family shows an autosomal dominant mode of inheritance. The only effective treatment for the chronic cysts and sinuses has been radical plastic

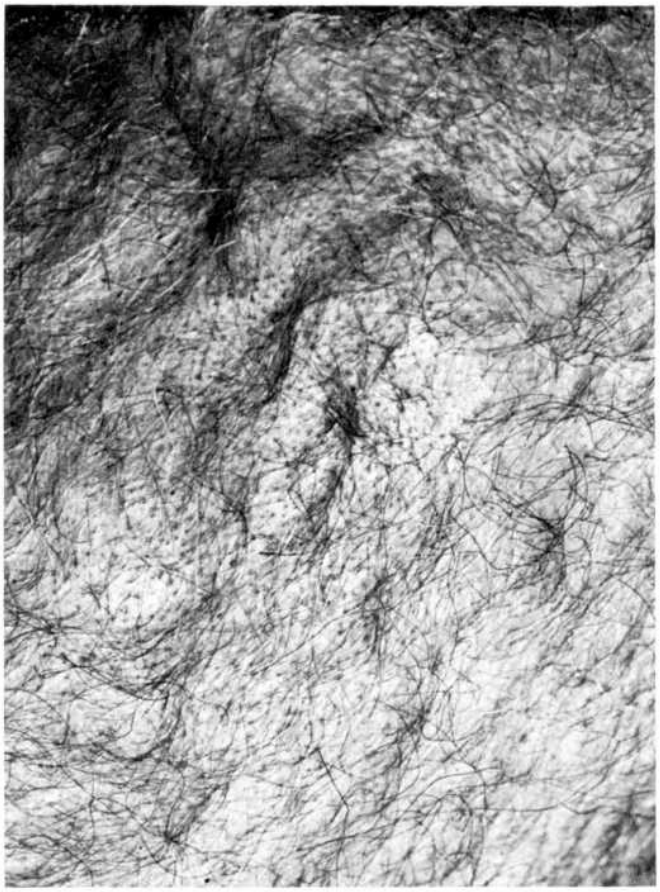

FIGURE 4. Scalp showing sparse twisted hairs.

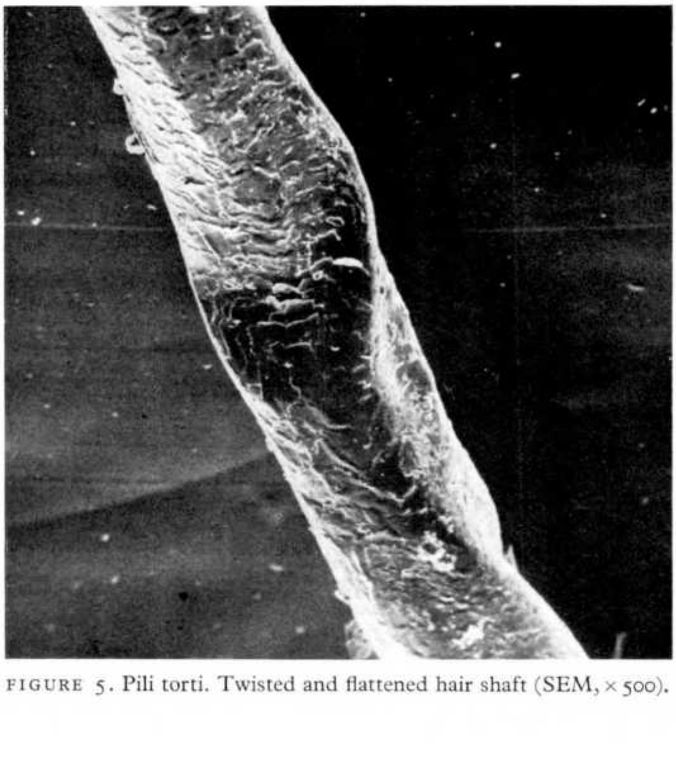

FIGURE 5. Pili torti. Twisted and flattened hair shaft (SEM, × 500).

surgery. As squamous carcinoma is a well-described complication of hidradenitis suppurativa (Humphrey, Playforth & Leavell, 1969), further surgery is contemplated both to reduce this risk and to relieve his present symptoms.

ACKNOWLEDGMENT

We would like to thank Dr J. A. Swift of Unilever Research Laboratories, Isleworth, for the electron microscope photograph.

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(12) Psoriasis with arthropathy, treated with methotrexate for 9 years

DR R.CLAYTON FOR DR M.FEIWEL

St Mary's Hospital, London

L.P., male, aged 43 years. Laboratory technician. Case No. J3607.

History. He has had psoriasis for 22 years. He was started on methotrexate in 1965 after being bed-ridden with acute arthropathy. Initially he received 2·5 mg daily, and from 1968 to January 1972, oral or intramuscular doses every 1–3 weeks (8–25 mg weekly) titrated according to the severity of the psoriasis. He was restarted in May 1972 following a severe continuous relapse, and is presently on 15 mg weekly by mouth.

He had tuberculous cervical glands in 1964, treated with antituberculous therapy for 18 months. There is no clinical evidence of recurrence.

Investigations. Chest X-ray normal. Full blood count always normal. Liver function tests always within normal limits, except for bromsulphthalein test noticed to be raised once in 1971. Liver biopsy (January 1972), slight fibrosis and fatty change considered to be non-specific.

Comment. This patient illustrates the value of long-term methotrexate in psoriasis with arthropathy.

The response to methotrexate of the arthritis, initially unresponsive to analgesics, was dramatic. In spite of a past history of tuberculosis the conditions have been satisfactorily controlled with methotrexate for 9 years without complication.

(13) Pterygium: deformity of finger nails in association with generalized psoriasis

DR J.D.KIRBY AND DR D.D.MUNRO

St Bartholomew's Hospital, London

T.L., male, aged 65 years (retired).

History. He has had psoriasis for 44 years which started in his scalp and spread to involve the trunk and limbs, and a dystrophy of all finger and toe nails for 20 years. He has had two periods, of 6 months and 3 months, of intensive in-patient therapy with dithranol, coal tar preparations and ultra violet light.

Family history. His father had psoriasis.

On examination. He shows diffuse psoriatic scaling throughout his scalp, on his abdomen, back and limbs. There are scattered nummular patches on his chest. There is a dystrophy of all nails with pterygium formation, and the dorsal surface of the toes is involved with pustular psoriasis (Figs. 1 and 2). His buttocks previously treated with superficial X-ray show erythema and mild poikilodermatous changes.

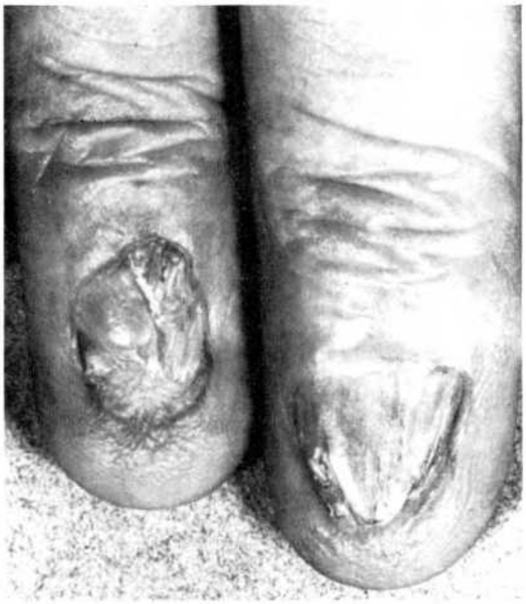

FIGURE 1. Showing dystrophy of finger nails.

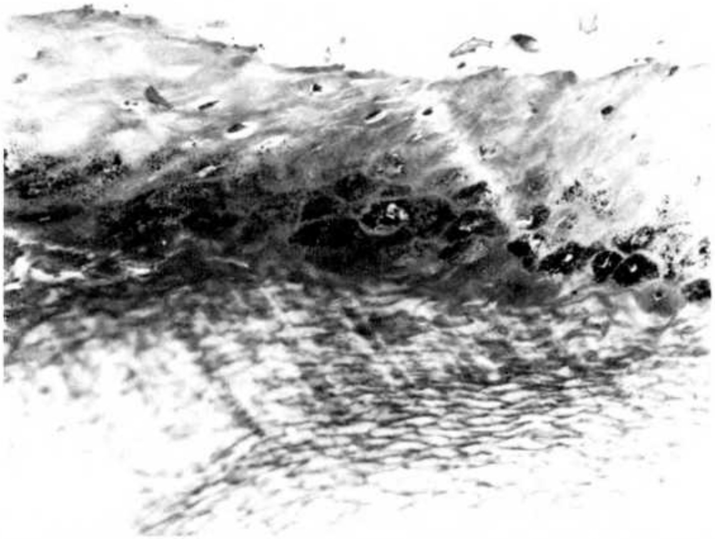

FIGURE 2. Nail plate and nail bed, showing presence of granular layer and nucleolysis (× 240).

Investigations. Haemoglobin, 13·7 g/100 ml; liver function tests normal; liver biopsy normal; longitudinal nail biopsy (25 February 1974) showed psoriatic involvement of nail bed and nail plate.

Treatment. Dermovate applied recently produced a widespread folliculitis. Azathioprine in an initial dose of 150 mg daily was started on 4 March 1974.

Comment. This type of nail dystrophy is associated with lichen planus. We are not aware that such changes in finger and toe nails have been described in psoriasis.

(14) Lichen myxoedematosus

DR R.W.GANGE FOR DR R.H. MARTEN

Kings College Hospital, London

A.W., male, aged 57 years. Hospital Case No. 64 74 89.

History. Itching, thickening of skin and hyperpigmentation have been noted since May 1973, becoming progressively worse (Fig. 1). He has had ankle oedema since November 1973 and herpes zoster in January, 1974.

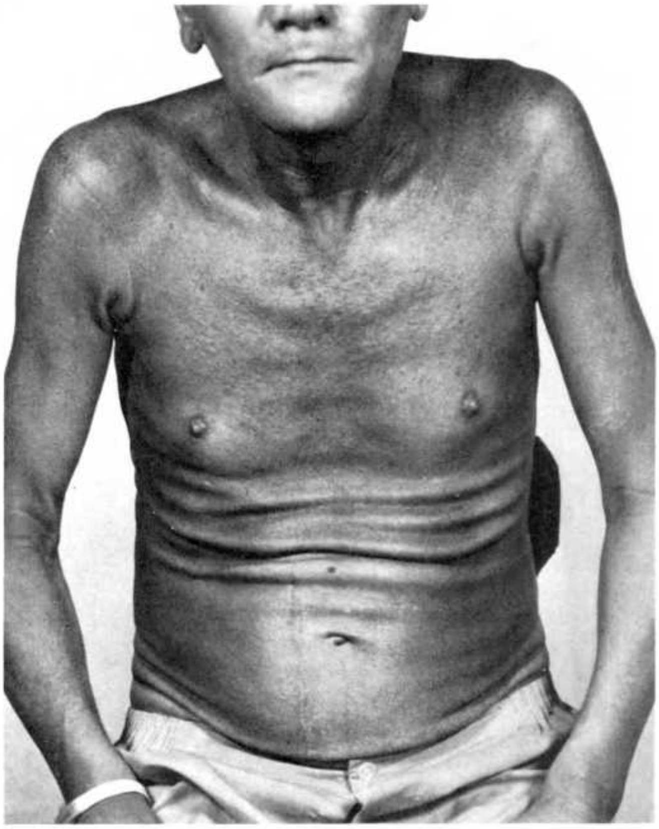

FIGURE 1. Thickening and hyperpigmentation of skin.

On examination. There is diffuse infiltration of the skin of his trunk, face, arms and thighs, with superimposed papular infiltration especially around his neck and behind his ears.

Investigations. Hb, 13·3; WBC, 10,900/mm^3; ESR 2; urea and electrolytes, normal; protein electrophoresis, faint monoclonal band in gamma region; no Bence-Jones protein; thyroid function, normal; chest X-ray, normal; barium swallow, some incoordination.

Treatment. None to date.

Comment. A typical case of lichen myxoedematosus, associated with small amounts of paraprotein. Furthur characterization of this is in progress.

(15) Klippel–Trenaunay syndrome

DR SUZANNE ALEXANDER
Barking Hospital

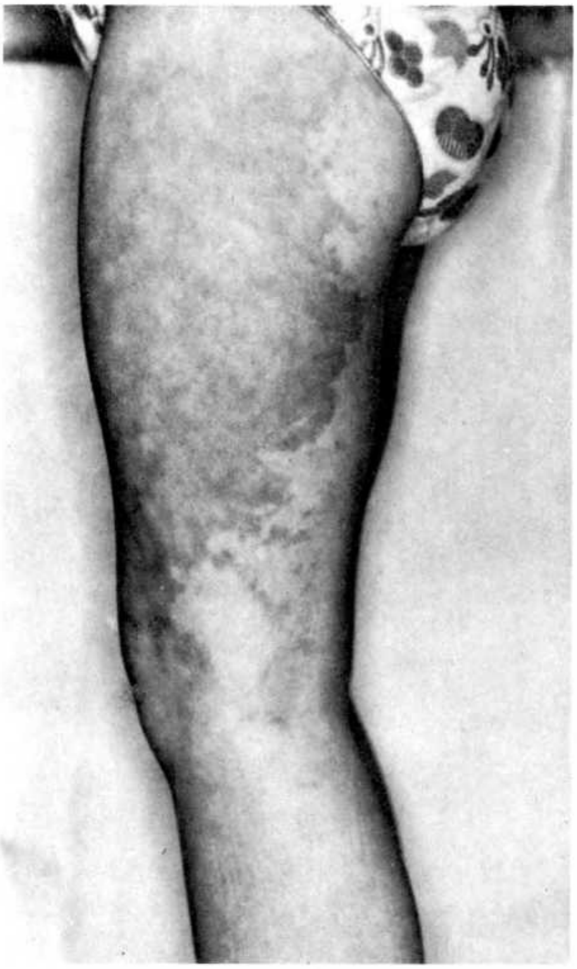

FIGURE 1. Capillary angioma on left thigh.

A.F., female, aged 3 years. Hospital case no. 44872.

History. She was noticed to have a reddish patch on the left leg shortly after birth. The left leg is thinner than the right, but there is no increase in length.

On examination a pale capillary angioma is present on the left leg with wasting of the quadriceps (Fig. 1).

Investigations. X-ray shows muscle wasting and decrease in subcutaneous fat. Bone texture normal.

(16) Klippel–Trenaunay syndrome

DR SUZANNE ALEXANDER

Barking Hospital

P.A., female, aged 3 months. Hospital case no. 61956.

History. A 'birthmark' was noticed on her right forearm at birth.

Family history. She has two brothers aged 4 and 3 years who are well.

On examination there is a huge strawberry naevus involving the whole of the right forearm, upper arm and hand, of mixed capillary and venous type, tending to ulcerate in fissures (Fig. 1).

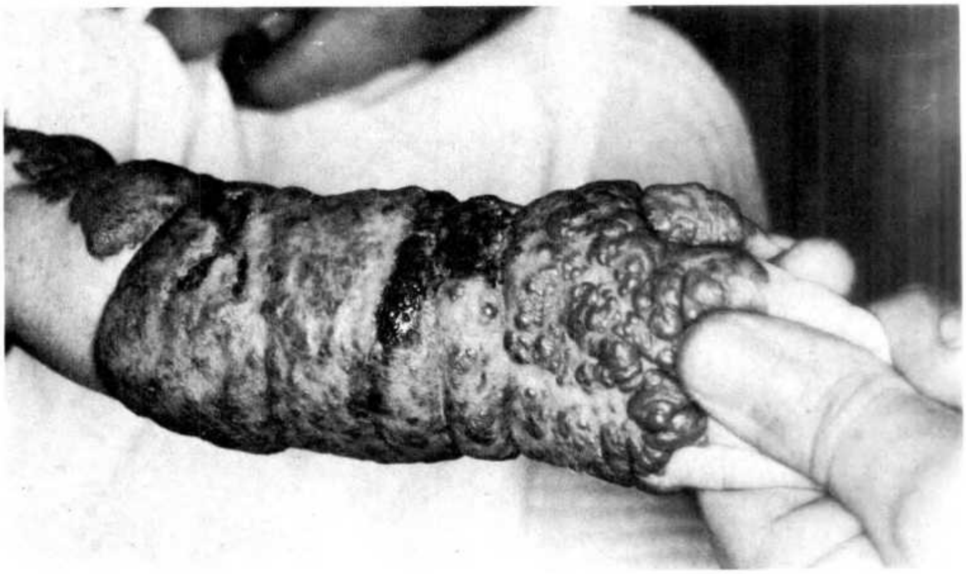

FIGURE 1. Angioma on right forearm.

Investigations. Normal blood count and adequate platelets. X-ray shows some streaky translucencies within the bones of the forearms, of both radius and ulna, suggestive of angiomatous change.

(17 and 18) Rothmund–Thomson syndrome

DR SUZANNE ALEXANDER

Barking Hospital

J.T. and A.T., females, aged 4 and 2 years. Hospital case nos. 41071 and 58430.

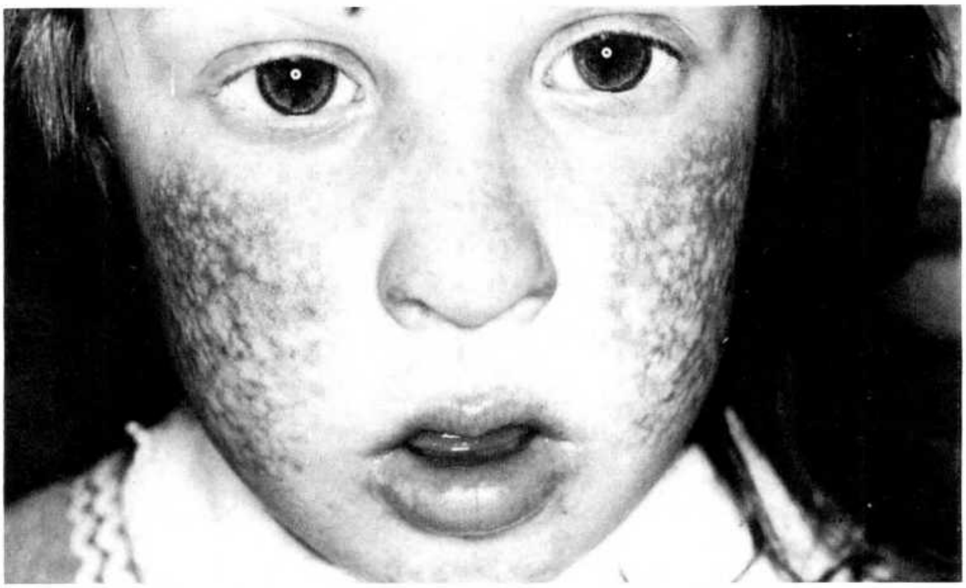

FIGURE 1. J.T. Poikilodermatous changes on face.

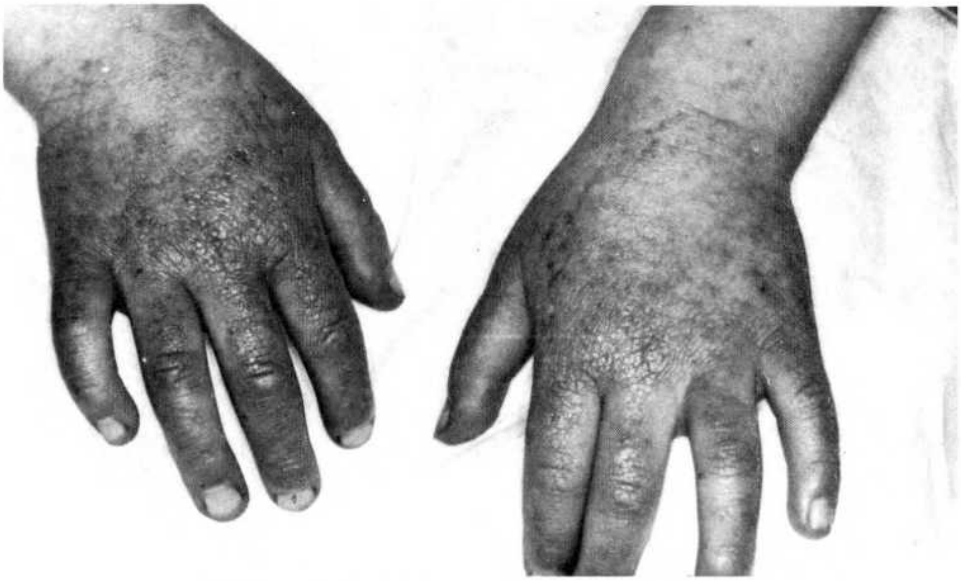

FIGURE 2. J.T. Poikilodermatous changes on hands.

History. Both these two sisters developed erythema of the cheeks at 2 months, gradually spreading to the chin, hands, wrists, nose, arms and knees.

Family history. Two brothers aged 9 and 8 years are normal.

On examination both children show reticulate erythema of the cheeks, chin, nose, hands and knees (Figs. 1–4). No evidence of cataract.

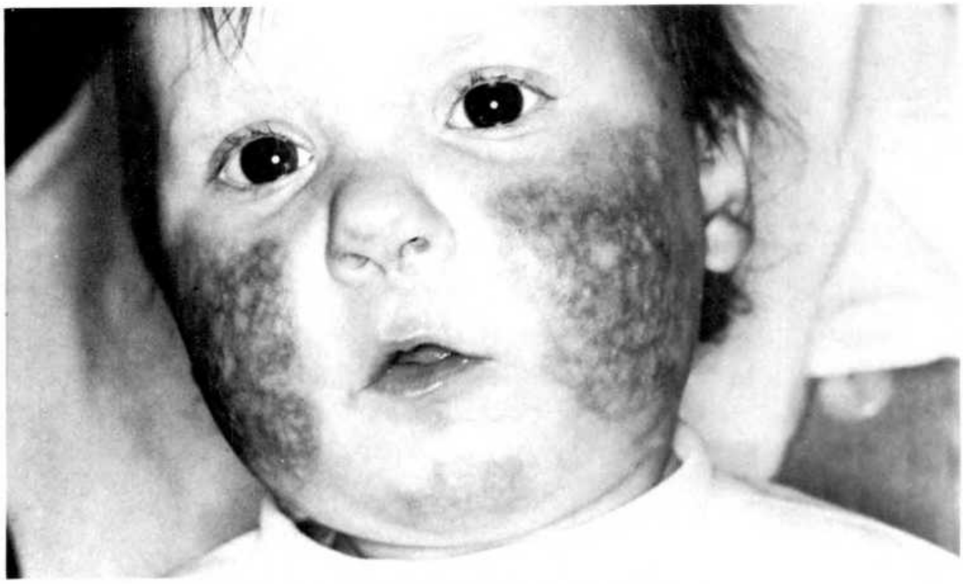

FIGURE 3. A.T. Poikilodermatous changes on face.

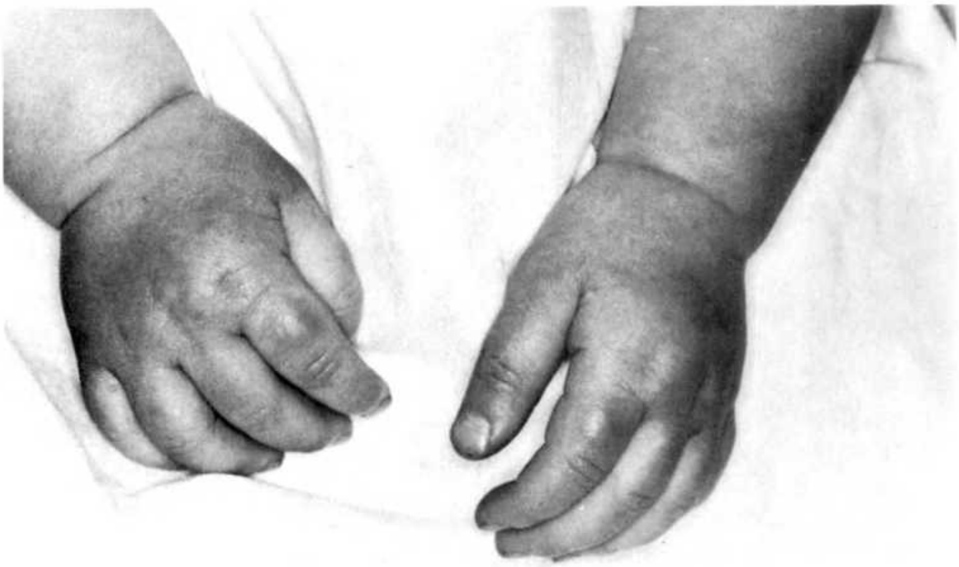

FIGURE 4. A.T. Poikilodermatous changes on hands.

Investigations. Monochromator testing at 300–320 nm—normal (Professor Ian Magnus and Dr Colin Ramsay). 'Porphyrins' normal. Fibroblast culture showed normal pair synthesis after u.v. irradiation (Dr Gianelli).

Treatment. Only sun protection creams have been used.

(19) Pigmented naevus of chest

Dr J.A.Carruthers for Dr P.F.D.Naylor

St Thomas' Hospital, London

K.M., female, aged 1 year. Hospital Case No. 58 32 57.

History. A pigmented area in the right pectoral region has been present since birth. There has been no increase in the area of skin involved.

On examination. An area of skin in the right pectoral region shows irregular pigmentation (Fig. 1). The surrounding skin is red and gives a geometrical outline to the involved skin.

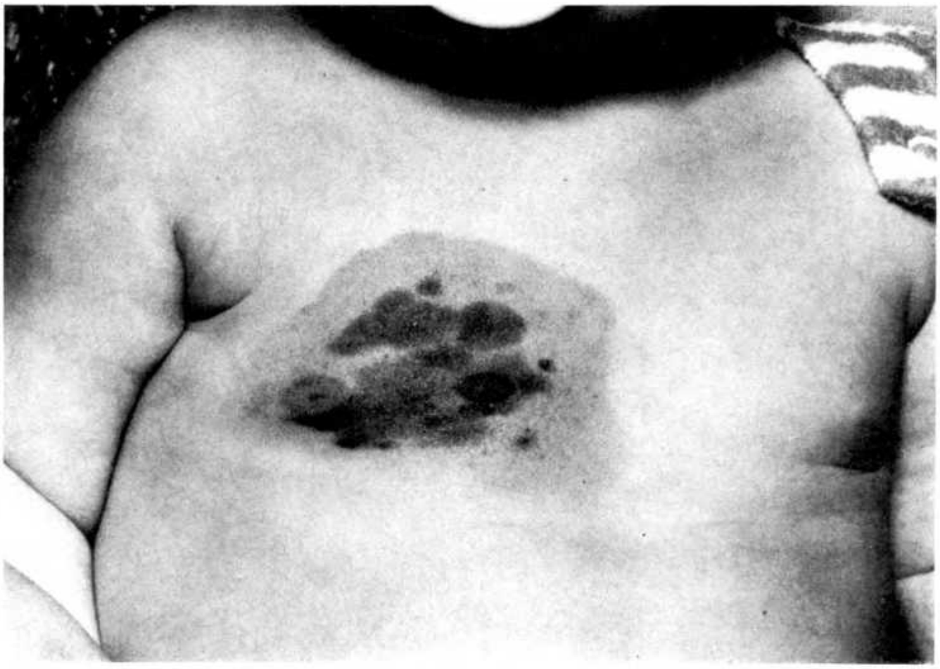

FIGURE 1. Irregular pigmentation with surrounding zone of erythema.

Some areas of pigmentation have gradually increased in intensity and new areas have appeared, but within the original outline. The erythema has become rather less obvious.

Serial u.v. photographs are available.

20) Granuloma of the finger due to injection of Freund's adjuvant

DR P.J.AUGUST FOR DR H.J.WALLACE

St Thomas' Hospital, London

H.C., female, aged 29 years.

History. The patient is a 29-year-old doctor who presented in May 1973 with pain and swelling of the pulp of the left index finger following the accidental injection of Freund's adjuvant with added rabbit anti-human IgM and human IgM. The accident took place during an experiment when an assistant failed to hold the test animal, a rabbit, sufficiently firmly. The point of the needle scarcely entered the finger pulp. The lesion bled copiously. The needle was essentially sterile but had been used on another rabbit. Within 2 h there was considerable pain and some swelling of the digit (Fig. 1). The patient was given a tetanus toxoid booster and clindamycin 150 mg four times daily. The finger improved somewhat over the next 2 days.

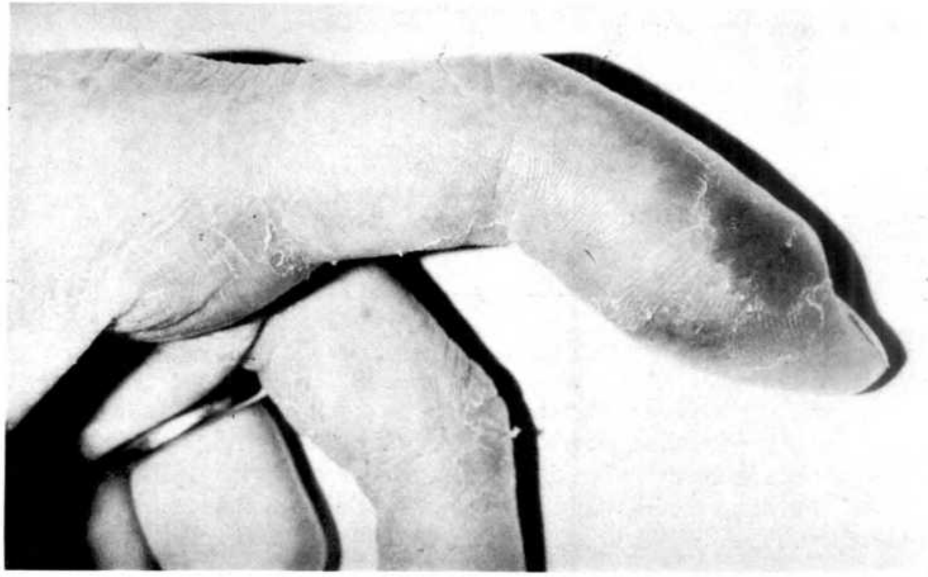

FIGURE 1. Granuloma on finger after injection of Freund's adjuvant.

One week later an influenza-like illness, with lower abdominal colic and diarrhoea, developed for 2 days. The clindamycin was stopped. Within 2 days the finger became worse again and a generalized maculo-papular rash developed, so treatment was continued with erythromycin. The rationale for this lay in the resemblance of the dull erythema to erysipeloid which is caused by *Erysipelothrix*, a known commensal of rabbit fur.

The entire finger, as far as the knuckle, became purple–red in colour and sausage-like in shape, with confluent softening of the radial side of the pulp. No tenosynovitis, ulceration, lymphangitis, lymphadenopathy or pyrexia developed.

Investigations: Hb, 13.2 g/100 ml; WBC, 5800 mm^3; ESR, 8; immunoglobulins, normal; X-ray of finger, normal, although later osteoporosis developed in the terminal phalanx; RA latex test, negative.

Course: Slow resolution of the swelling took place during the following months, although the finger tip remained tender to pressure. Recovery was interrupted in August 1973 by an attack of ophthalmic herpes zoster. After 7 months a small pocket of pus formed in the joint crease of the distal interphalangeal joint. The aspirate from this was sterile on Lowenstein's medium. Some pulp atrophy, reduction in movement at the distal interphalangeal joint and tenderness to deep pressure in the distal phalanx have persisted.

Discussion: This would appear to be a granuloma provoked by the challenge of a mixture consisting of immunoglobin IgM, antibody to the IgM, and tuberculo-protein. The particular Freund's adjuvant used contained: paraffin oil 85%; mannide mono-oleate (an emulsifyer), 15%; *Mycobacterium butyricum* (killed and dried), 0.05%.

The subject was Mantoux positive in childhood. Immunologically she seems normal in all respects. She had an ampicillin rash during an attack of glandular fever in 1967. She had a rash attributed to clindamycin during the onset of the digital granuloma. She showed a normal rise in antibodies to VZ virus during her attack of zoster. There is no family or personal history of auto-immune disease. Despite being immunized with IgM she did not develop a serum rheumatoid factor. Understandably, the patient is reluctant to undergo intradermal testing with any of the agents present in the initial injection at the time of the accident.

This case has been reported as there has been a substantial morbidity from the injury. Casualty officers and dermatologists are likely to see more cases now that many hospital laboratories are using Freund's adjuvant in animals to raise antibodies for immunochemical determinations.

(21) Benign Hyperglobulinaemic Purpura

DR R.C.D.STAUGHTON FOR DR G.C.WELLS

St Thomas' Hospital, London

S.B., female, aged 49 years.

History. She gives a 33 year history of recurrent showers of purpura, chiefly on the lower limbs but occasionally under areas of clothing pressure, at sites of trauma, and on the arms. Orthostatic lesions commonly appear late in the day, often following exertion, prolonged standing or 'spontaneously'. Purpura is consistently more profuse when she is 'overwrought' or worried. Lesions are preceded by local itching and redness. There has been a gradual accumulation of dark brown pigment from toes to knees (Fig. 1).

Attacks are not associated with arthralgia or abdominal pain; there is no history of abnormal bruising, bleeding or Raynaud's phenomenon.

Approximately once a year, she experiences a 'flu like' illness of varying severity but similar pattern. This begins with headache and malaise, and then proceeds to marked arthralgia and myalgia. She feels febrile and develops red blotches over her whole body. She remains bedridden for about a week and continues to feel lethargic for two more.

In May, 1972 she had an episode of *E. coli* septicaemia during which there was conclusive laboratory evidence of a consumptive coagulopathy but no overt bleeding. Following her recovery there was a mild temporary glucose intolerance.

Family History. Her sister died in New Zealand with 'cancer in the eye' and myocardial infarct.

On Examination. There are purpuric lesions 1–3 mm across scattered from buttocks to toes, on her shoulders, under her brassiere straps and in a scratch mark on her thigh (Köbner phenomenon).

There is dark brown pigmentation from feet to knees, and her skin is dermographic. There is no lymphadenopathy, splenomegaly, xerostomia, or parotid enlargement.

Investigations. Hb 12.4 g/100 ml. WBC 2400 mm^3 (neutrophils (864) 36%, lymphocytes 59%). Platelets 189,000 mm^3. ESR 70 (1972), 49 (1974). Serum iron 0.55 mg/l (3.07–3.5), i.e. chronic disease pattern. Latex titre 1 in 320. Rose-Waaler titre 1 in 512. Antinuclear factor 1 in 250. Total proteins 8·1 g/100 ml (raised). Albumin 4·2 g/100 ml (normal). Electrophoresis; diffusely increased gamma globulin. IgG 3080 mg/100 ml (raised); IgG 244 mg/100 ml (normal). IgM 96 mg/100 ml (normal). LE cell preparation; negative × 3, occasional LE cells seen in one preparation. Anti-DNA antibody; within normal limits.

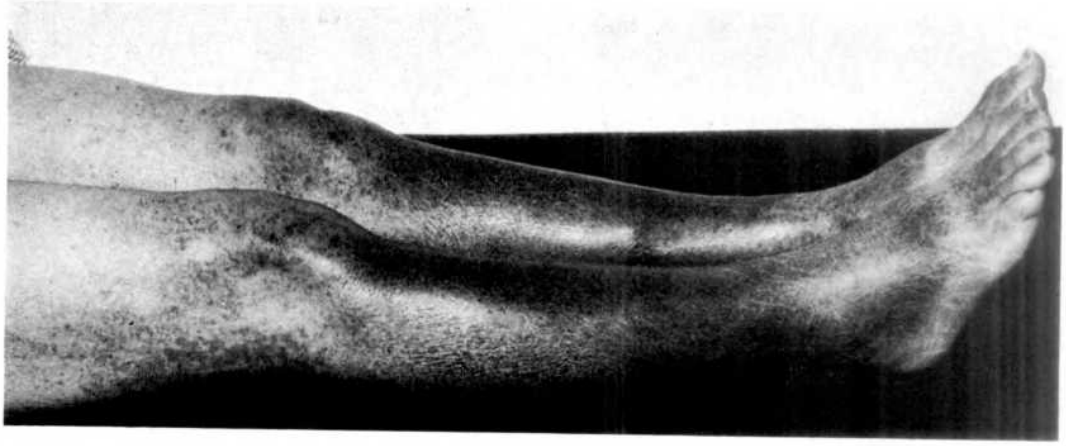

FIGURE 1. Dark brown pigmentation from feet to knees.

Normal investigations. Bone marrow, WR, cryoglobulins, cold agglutinins, Coombs' test, antibody screening, clotting time, complement, platelet function, renal function, liver function and urine. Direct immunofluorescence staining failed to show the presence of immunoglobulins (IgG, M, or A) or complement C_3 in tissue. A single dermal vessel demonstrated fibrin deposition in its walls. Hess' test; negative. Scratch test; exaggerated triple response, no purpura. Subcutaneous injection gave no effect with either 0·5 ml of her own serum or own whole blood.

Comment. Hyperglobulinaemic purpura (HP) is a syndrome, first described by Waldenström in 1943, comprising recurrent orthostatic purpura, an increased sedimentation rate and elevated serum globulins. It occurs in otherwise well adult females. It is fairly easily differentiated from either Waldenström's macroglobulinaemic purpura (a chronic illness of old men with lassitude, mucosal bleeding, and frequent cerebral and retinal haemorrhages, characterized by monoclonal hypergammaglobulinaemia of the IgM class); or from cryoglobulinaemia of Lerner and Watson (where acral purpura is usually associated with ecchymoses and gangrene and the serum contains a paraprotein precipitating in the cold.)

When a collection of patients with HP are followed for any length of time, approximately half develop other conditions, for example Sjögren's syndrome, SLE, lymphoma, sarcoidosis or multiple myeloma. These are then designated secondary hyperglobulinaemic purpura, the residue being 'essential' or benign hyperglobulinaemic purpura (BHP). Careful prospective analyses have failed to reveal any laboratory tests discriminating between the two groups, but most secondary cases begin after the age of 40 years and are less exclusively female.

Several excellent series attest the chronicity of BHP (often 10–20 years) and have further elucidated the serum abnormalities. Electrophoresis demonstrates diffusely raised gamma globulins, chiefly IgG material. Ultracentrifugation of this fraction reveals fast moving (7S) intermediate auto-immune complexes between gamma globulin and IgG anti-gamma globulin. Rheumatoid factor activity and anti nuclear factor are almost always positive.

The pathological process underlying these globulin complexes is obscure. Kyle *et al.* (1971) noting the chronicity, female preponderance, ANF, and rheumatoid factor, suggested that BHP is a facet of the collagen diseases. Waldenström makes the further hypothesis that such patients have a constitutional ability to form antigamma globulin too easily. For years, they may have slightly elevated globulins, micro-adenitis and slight anaemia, with or without purpura. Some, when challenged with certain antigens or haptens, for example penicillin or butazolidine, react with allergic manifestations or severe symptoms like those of SLE. He quoted a case of BHP in which aggressive episodes of SLE were twice precipitated by butazolidine treatment. It would therefore probably be wise to withold such antigens from patients with BHP (namely: chlorpropamide, griseofulvin, hydrallazine, hydantoins, isoniazid, PAS, penicillin, phenothiazines, phenylbutazone, procainamide, sulphonamides and thiouracils).

The pathogenesis of the purpura is also obscure. Hydrostatic pressure must be important, and raised serum viscosity may give rise to capillary sludging, micro-infarction and the extravasation of blood. The early symptoms of burning and itching may be caused by a local Arthus reaction.

Treatment is disappointing but in most cases unnecessary. In our case, purpura was abolished by bed rest, also support stockings and leotards have been useful in cases where discomfort was troublesome. Steroid therapy has usually been unhelpful. Cytotoxic drugs (chlorambucil and thioguanine) have been reported to decrease purpura, but they are toxic and do not appear to alter the electrophoretic pattern.

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(22) Osteoma cutis possibly arising in old acne vulgaris*

DR T.J.DELANEY AND DR S.C.GOLD

St George's Hospital, London

W.M.McR., male, aged 53 years.

History. When aged about 35 years, he noticed symptomless hard lumps developing on the anterior part of his chest. These continued to increase in size and number for approximately 10–15 years. Many have been removed as they catch on clothing, and for cosmetic reasons.

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* Previcusly shown at the meeting of the Section of Dermatology of the Royal Society of Medicine, 18 June 1964.

He has also developed numerous superficial basal cell epitheliomata which may be related to sun exposure. There is no family history of either skin condition, nor is there a history of arsenic ingestion. He had acne as a teenager, but not of severe degree.

On examination (1974) there are a number of hard nodules on the anterior aspect of his chest, mainly in the presternal area (Fig. 1). Scars are present from removal of some of these lesions and also from the treatment of scattered superficial basal cell epitheliomata.

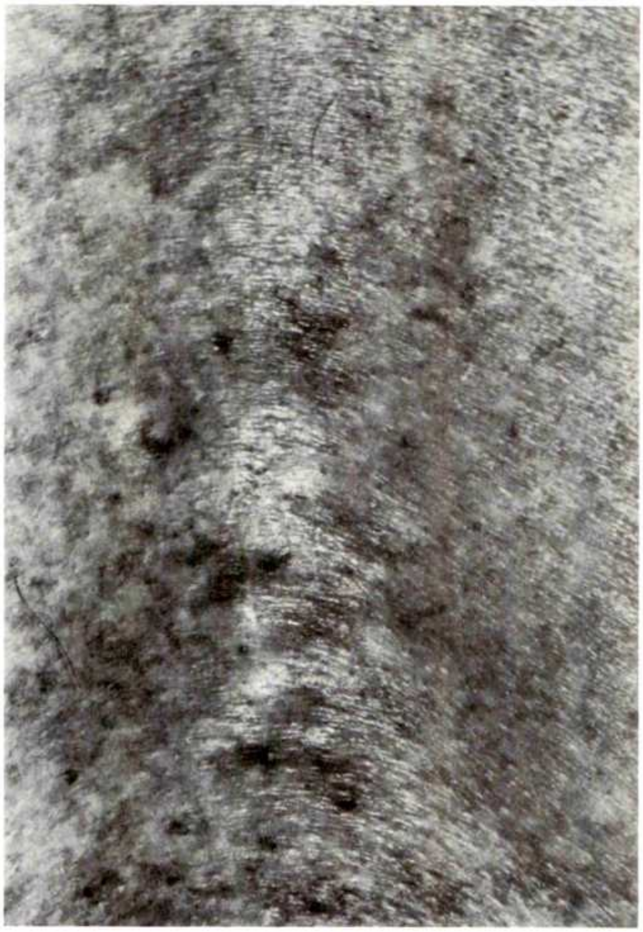

FIGURE 1. Numerous small subcutaneous nodules in presternal area.

Invesigations. Histology—all of the nodules examined have shown osteoma cutis (Fig. 2) with no evidence of a preceding cause. The clinical diagnosis of basal cell epithelioma has been confirmed for all of these lesions removed (Fig. 3). Chest X-ray (Figs. 4 and 5) confirms the presence of calcification in the nodules. X-ray of abdomen—no abnormal calcification. Serum calcium, phosphate and alkaline phosphatase—normal.

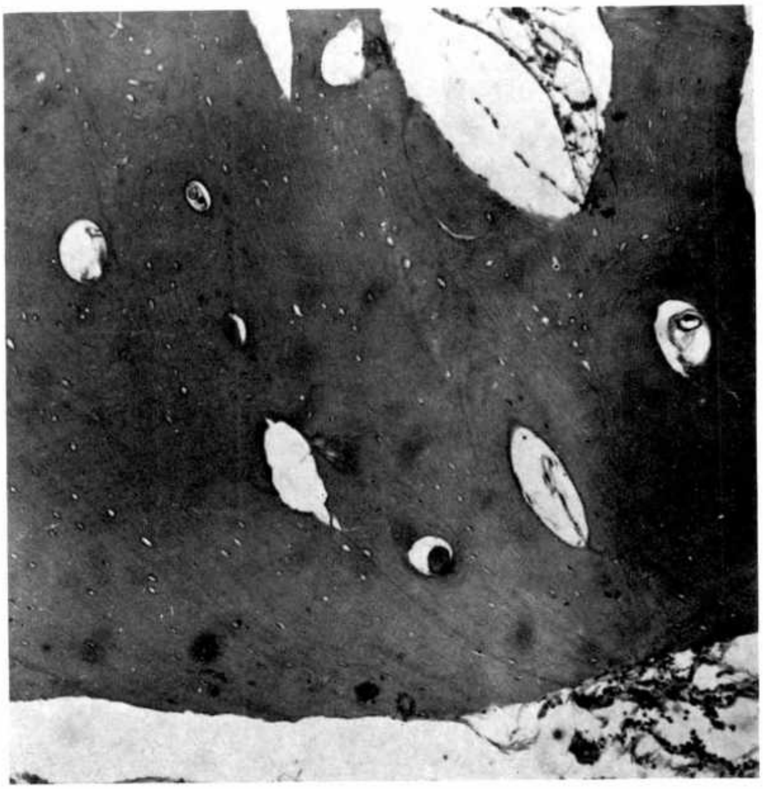

FIGURE 2. Osteoma cutis showing typical mature bone formation (decalcified, H&E, × 90).

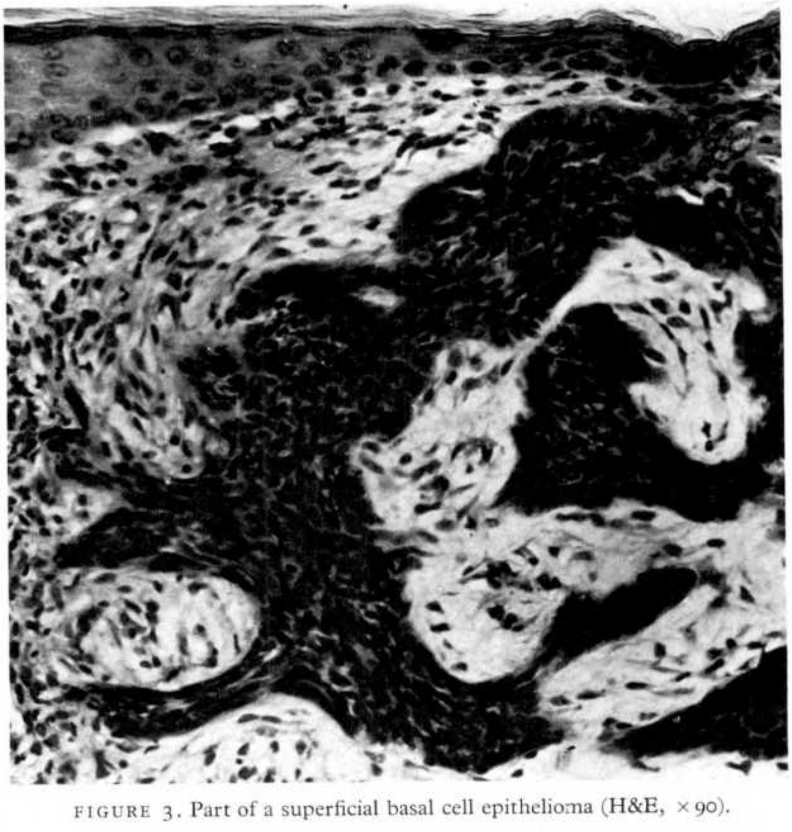

FIGURE 3. Part of a superficial basal cell epithelioma (H&E, × 90).

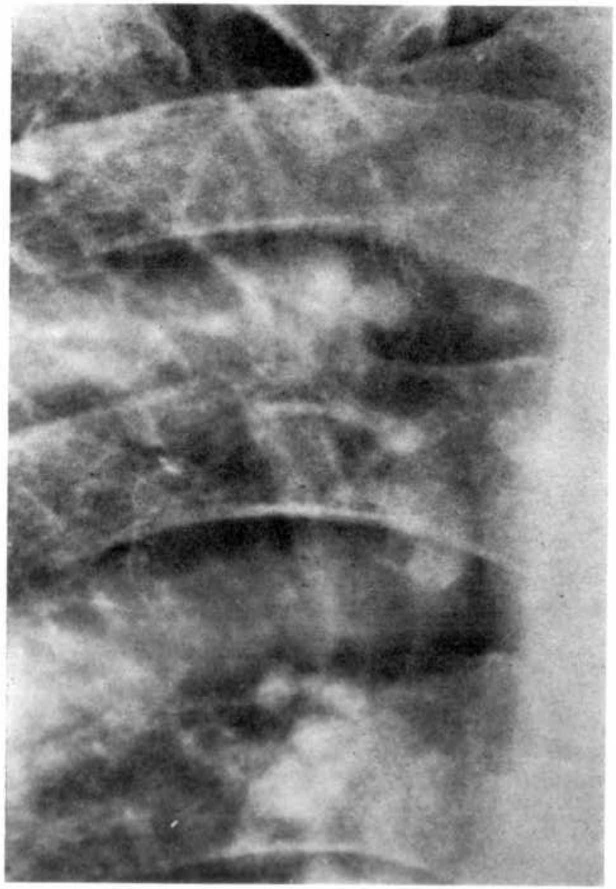

FIGURE 4. Chest X-ray showing numerous opacities in the right lung field.

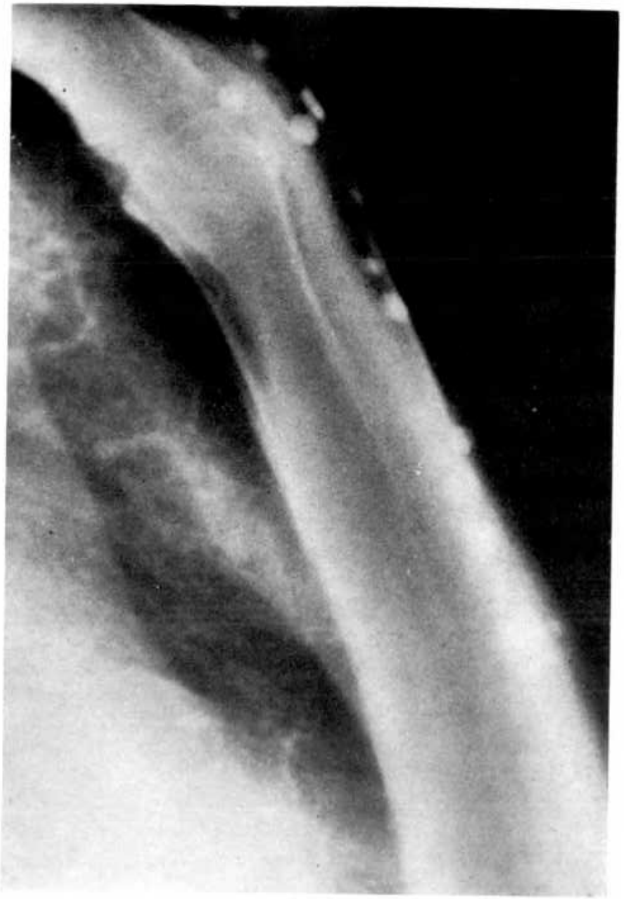

FIGURE 5. Chest X-ray—lateral view confirms the opacities to be superficial.

Comment. The site of the ossification in this patient, together with the absence of any other relevant disease, would indicate that this is an example of calcification occurring in acne scars or cysts (Sanderson, 1972). Perhaps the most important aspect is to realise that this form of ossification can enter the differential diagnosis of shadowing on an X-ray (MacGregor, 1971).

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(23) Anderson-Fabry disease (AFD)

DR H.J.WALLACE

St Thomas' Hospital

Case (a) A.B., male, aged 20 years. Student. Hospital No. 49 64 32.

History. The early changes in this family were reported by Flynn *et al.* (1972). The onset of his illness was at the age of 8 with burning pains in the feet. Similar pains began in the hands at the age of 15. Attacks last from minutes to days and may be accompanied by fever and a raised ESR. They may occur spontaneously but are most readily caused by heat or exercise. They are partially relieved by pressure. Since the age of 15 he has had attacks of abdominal pain which may last for several days. He has had secretory otitis media since early childhood.

Family history. See Fig. 1.

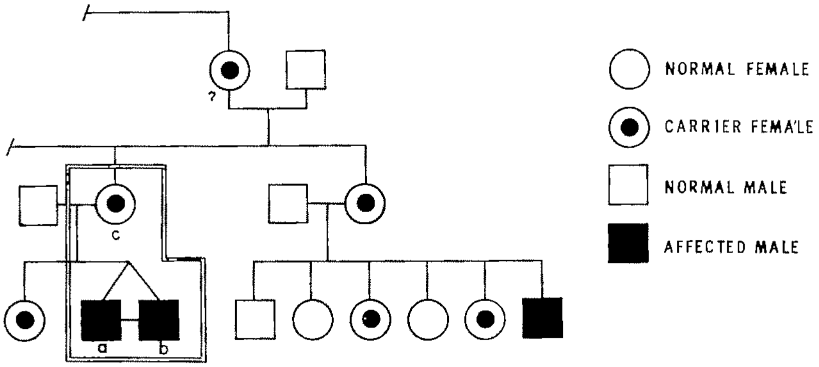

FIGURE 1. Family history for cases (a), (b) and (c); showing carrier females and the affected male progeny.

On examination. There are a few small angiectases, some arranged in groups, mainly over the scapulae but also on the outer aspect of the upper arms, and around the umbilicus. There is gross dilatation and tortuosity of the retinal veins. Slit lamp examination of his corneas shows general haze and superficial opacities extending from the limbus to a point just below the centre of the cornea. Both eardrums are retracted. He has a slightly clumsy gait with a tendency to walk on the outside of his feet.

Investigations. Blood. Abnormal, α galactosidase activity in leukocytes (Kint, 1970) was diminished being 8 μmoles hydrolysed/mg protein/h (normal 42·7–65·6); ESR raised intermittently; occasional eosinophilia.

Normal, Hb; WBC; film; electrolytes; urea; proteins including lipoproteins; liver function tests; blood group Xg^a.

Urine. Occasional albuminuria. Spun deposits, no birefringent lipid globules. Electron microscopy; lamellar bodies consistent with AFD and preceding onset of albuminuria (Wallace, 1973). Chromatography; excess ceramide dihexoside and ceramide trihexoside.

X-ray. Barium meal; the jejunum is dilated with transverse barring, and there is some widening of the ileum. Barium enema; the descending colon and distal half of the transverse colon are narrow, tubular, and devoid of haustration.

Biopsy. Jejunum; the villous pattern and α-galactosidase activity are normal. The neurones of Meissner's plexus are enlarged with birefringent vacuolation, staining positively with Sudan Black and Luxol Fast Blue. Similar changes are found in the media of blood vessels and in muscle cells of the muscularis mucosae.

Course and treatment. There has been significant relief of pain with diphenylhydantoin up to 100 mg daily (Lockman *et al.*, 1971).

Comment. He shows the following features: few angiectases; increased urinary excretion of ceramide dihexoside and ceramide trihexoside; gross diminution of α-galactosidase in leukocytes; characteristic electron microscopic appearances in urine deposit which preceded any albuminuria.

Case (b) D.B., male, aged 20 years.

History. This is similar to that of his twin brother, case (a), but he seldom gets abdominal pain.

On Examination. He shows a few small angiectases mainly over the scapulae and outer aspect of his upper arms.

Investigations. The results are essentially the same as for case (a), but no radiological changes were detected in the gut.

Treatment and comment. Similar to case (a).

Case (c) M.B. female, aged 47 years. Artist. Hospital number 51 89 98.

History. She is the mother of Cases (a) and (b), and is a known carrier of AFD. In 1972 sterilization was performed at her own request. Total hysterectomy, with bilateral salpingectomy and wedge resection of the right ovary, was followed a few weeks later by the onset for the first time of attacks of indescribable burning pains in the feet. These attacks lasted for several months. Her general health is good.

On Examination. She has no significant angiectases. There is tortuosity of the retinal veins. Slit lamp examination of the corneas shows faint linear opacities extending from the limbus to a point just below the centre of the cornea. Her colour vision is normal.

Investigations. Investigations as for cases (a) and (b) were normal, including normal α-galactosidase activity in leukocytes. Blood group Xg^a.

Biopsy. Uterus, salpinges and ovary show vacuolation in the media of blood vessels, staining positively with Luxol Fast Blue. Electron microscopy shows lamellar bodies consistent with AFD.

Comment. She shows the following features: symptomless before her operation; no significant angiectases; characteristic eye changes; normal α-galactosidase activity in leukocytes.

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(24) Porphyria cutanea tarda

DR P.J. AUGUST AND DR G.C. WELLS

St Thomas' Hospital, London

J.D., male, aged 51 years.

History. He was referred in May 1972 by his general practitioner because of the development of pale, thickened areas in the skin of the light exposed areas of the face and neck (Fig. 1). He was lost to follow-up until January 1974 when he presented via his general practitioner with a 6 week history of malaise, post prandial epigastric pain and vomiting. He admitted to consuming 8 pints of beer daily, an intake which had, in the past, been 20 pints.

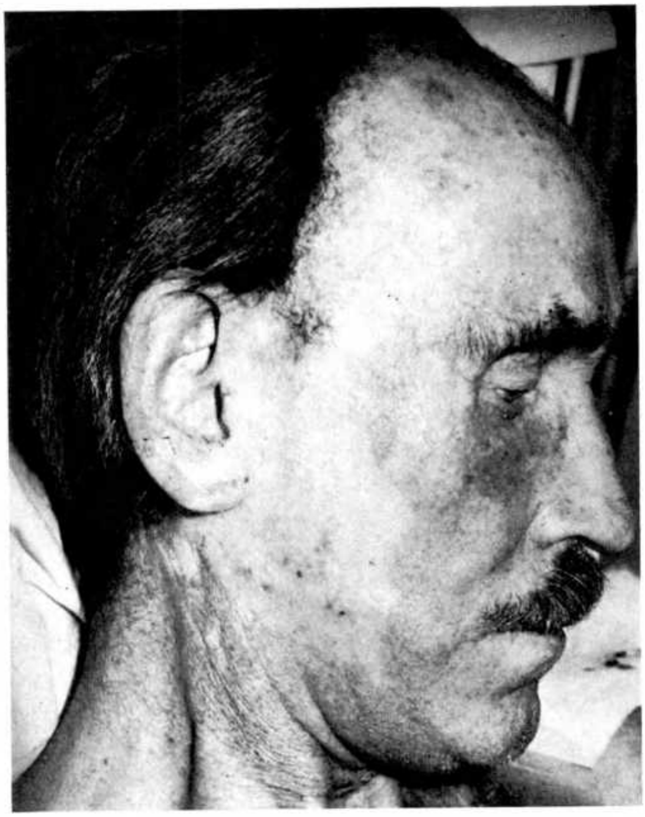

FIGURE 1. Porphyria cutanea tarda, sclerosis on face.

On direct questioning he confirmed that his skin would tear and blister on slight mechanical trauma. He was unaware of being particularly sensitive to the sun.

Examination. This revealed sclerodermatous changes confined to the light exposed areas, namely the frontal and vertical areas of the scalp (Fig. 2), the forehead, the 'V' of the neck (Fig. 3) and the backs of the hands. There was some pigmentation and a little telangiectasia. One erosion was seen over the left temporal area but there were no blisters. There was ichthyotic scaling over the back.

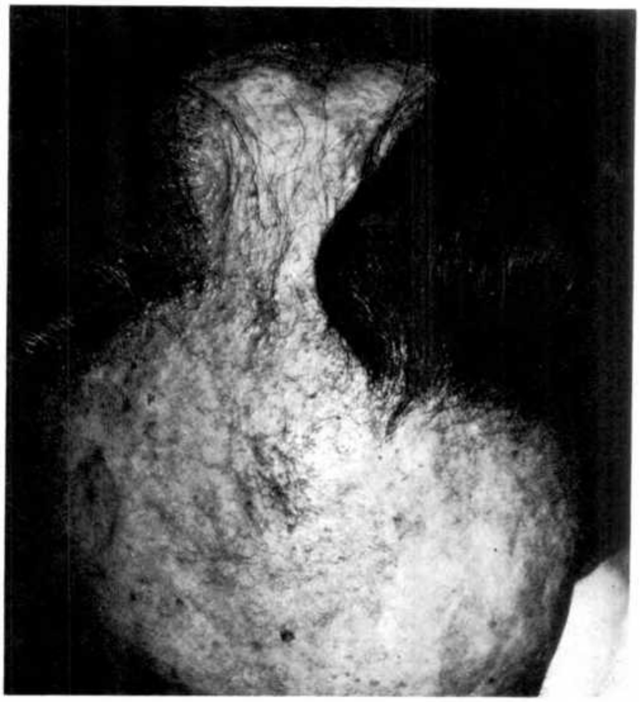

FIGURE 2. Porphyria cutanea tarda, sclerosis on scalp.

General examination. This showed the liver to be tender and irregularly enlarged with the lower edge reaching a hand's breadth below the costal margin. There were no other signs of hepatic disease.

Investigations. Hb 14·6 g/100 ml. WBC 5200/mm^3, ESR 23 mm/h (Westergren). SGOT 200 IU/l falling to 135IU/l after 1 week (upper limit of normal 65 IU/l). Alkaline phosphatase 21 KA units/100 ml (normal range 3–13 units). Bilirubin 1·2 mg/100 ml. Liver scan; the liver is enlarged and there are areas that may represent focal defects. Liver biopsy; the normal architecture has been lost. No normal lobules are seen and small clumps of liver cells are surrounded by fibrous tissue containing some inflammatory cells. The bile ducts look normal. The appearances are of active micronodular cirrhosis. Smooth muscle antibody; negative. Antimitochondrial antibody; negative. Antinuclear factor; negative. Alphafeto-protein; negative. Skin biopsy; there is very dense fibrous tissue which is causing thickening of the dermis. The adnexae are atrophic. The appearances are those of scleroderma.

Twenty-four hour urinary porphyrins: Coproporphyrin 130 μg/24 h (normal range 0–300 μg); Uroporphyrin 550 μg/24 h (normal range 0–40 μg). Chest X-ray; This shows sub-segmental atelectasis

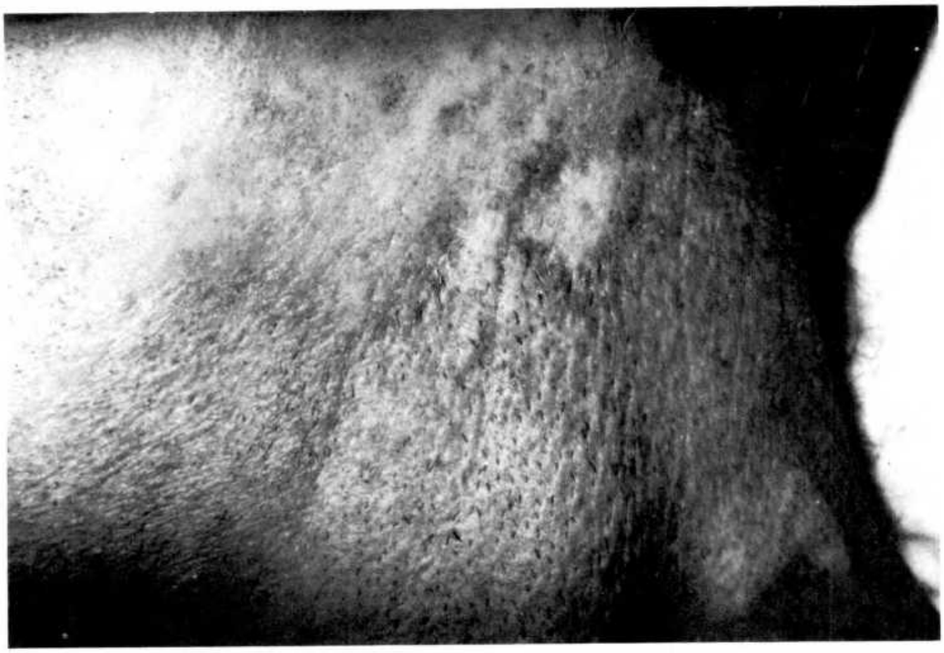

FIGURE 3. Porphyria cutanea tarda, sclerosis on neck.

at the right costo-phrenic angle. Barium swallow and meal; there is irregularity of the lesser curve surface of the pyloric antrum suggestive of ulceration. The duodenal cap is deformed and the postbulbar area may be ulcerated.

Course and treatment. His epigastric pain settled in hospital. He had failed to attend for follow up at the time of going to press, but treatment is going to be arranged.

(25) Cicatricial alopecia? Cause

DR R.C.D. STAUGHTON FOR DR G.C.WELLS

St Thomas' Hospital, London

E.J., female, aged 55 years. Machinist.

History. This 55-year-old Jamaican lady presented in December 1973 with a 5 year history of progressive hair loss and patchy depigmentation of the scalp. Her skin had otherwise been quite clear. She was in good health and had no relevant past history. On direct questioning she admitted to weekly straightening of her hair with hot combs for the past 20 years; the practice was continued until presentation.

On examination. The salient features were gross hair loss from the vertico-frontal area, with patchy depigmentation and some perifollicular hyperpigmentation (Figs. 1 and 2). Scarring was mild only. The mucous membranes, nails and the rest of her skin were clear.

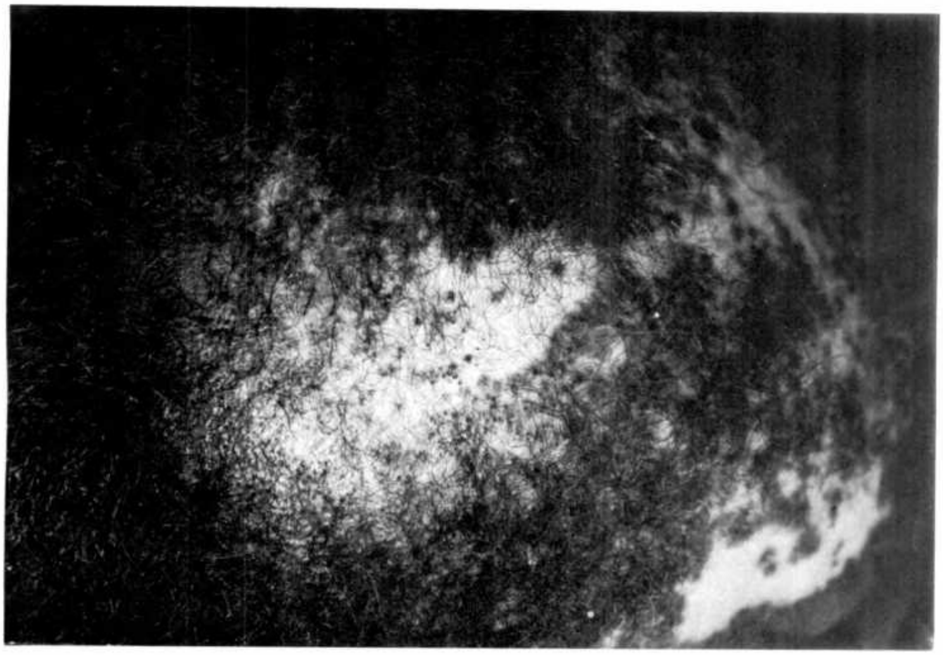

FIGURE 2. Vertico-frontal area of scalp, viewed from above

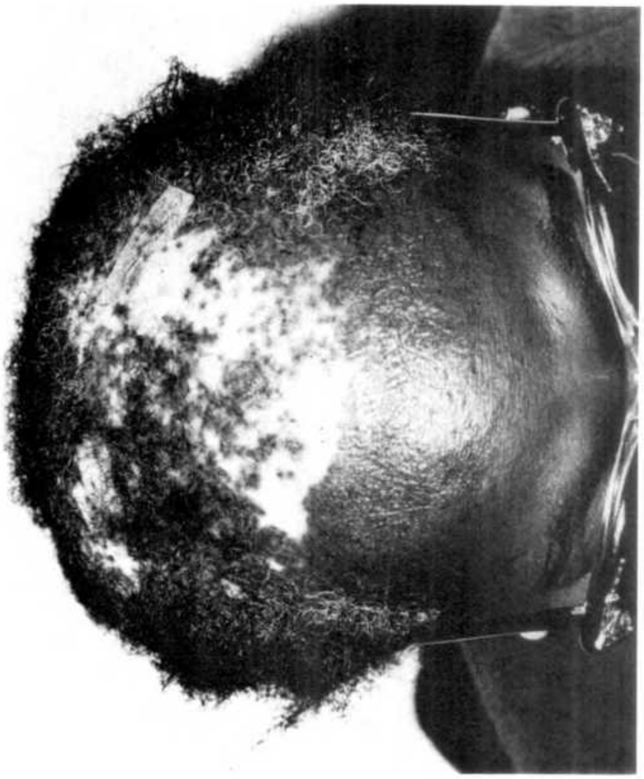

FIGURE 1. General view of scalp.

Investigations. Full blood count, W.R., and anti-nuclear factor were normal. Scalp biopsy: Hyperkeratosis of hair follicle mouths but no evidence of active lichen planus or other disease. Many hair follicles appear to have been lost completely.

Comment. The differential diagnosis includes lichen planus, discoid lupus erythematosus and 'hot comb alopecia'. The histological picture lends no support to lichen planus or discoid lupus erythematosus and is consistent with the late stage of hot comb damage. Depigmentation, however, has not been described in 'hot comb alopecia'.

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(26) Blepharochalasis

Professor C.D.Calnan

St John's Hospital, London

E.S., male, aged 21 years. Cleaner. Hospital No. P.35217.

History. For 4 years this man has complained of swelling of his upper eyelids. The degree of swelling varies but it does not subside completely. No other part of the face or lips is affected.

Family history. His mother has eczema, and his sister has psoriasis.

On examination. There is a diffuse swelling of the upper eyelids, the skin of which is so loose that it droops over the eyelashes.

Histology. Minimal oedema is present; connective tissue stains show no abnormality.

(27) Pigmented eczema and malabsorption

Dr J.A.Carruthers and Dr G.C.Wells

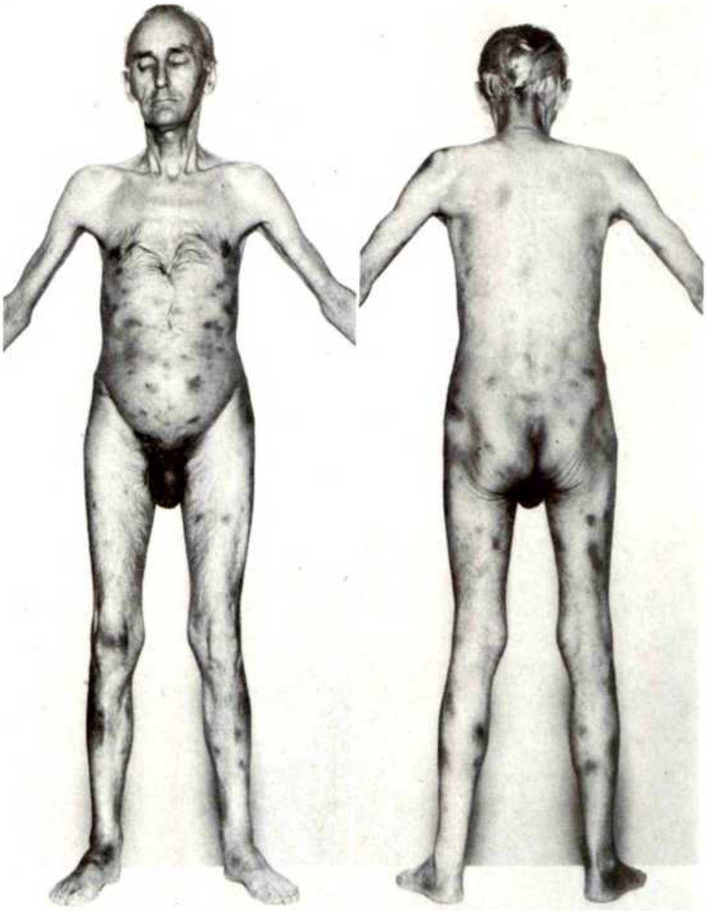
St Thomas' Hospital, London

H.D., male, aged 58 years

History. He first developed hand eczema in 1957. In 1966 he was admitted to St John's Hospital with vesicular eczema of the hands and feet with patchy spread on the body. Weight loss was attributed to pulmonary tuberculosis which was effectively treated.

He had a number of minor strokes between 1968 and 1970 which left him with pseudo-bulbar palsy. This was shown to be due to stenosis of both vertebral and the right carotid arteries. He had lost 3 stones over a few years and his dietary intake was poor. There was extreme lassitude but no alteration of bowel habit.

On examination. He was emaciated and depressed. Speech was slurred and swallowing was difficult. Bruits were audible over both carotid arteries. There was eczema of the hands, feet and legs, with scattered plaques on the thighs and trunk. These patches of eczema were dark brown (melanotic) (Figs. 1 and 2).



FIGURES 1. AND 2. Melanotic patches of eczema

Investigations. So far completed, the following abnormalities are noted; Hb, 8·2 g/100 ml a macrocytic, hypochromic anaemia: Marrow, megaloblastic with reduced iron stores; Serum iron, 0·1 mg/l (0·95–1·21); UIBC 2·9 mg/l (3·07–3·5); Serum B_{12}, 100 pg/ml (180–1000); RBC folate, 110 ng/ml (160–640); Serum folate, 1·9 ng/ml (5–20); Serum calcium, 8·2 mg/100 ml (9·0–11·0); proteins normal. Chest X-ray, 'gross distortion at the right apex possibly due to old tuberculosis'. Upper small bowel X-ray, abnormaly dilated with featureless mucosa. Stool fatty acid excretion 11·6 gm/24 h over 3 days.

Comment. Pigmentation of the eczematous lesions suggested an association with malabsorption. This type of case was reviewed by Wells (1962).

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(28) Pyoderma gangrenosum treated with azathioprine and prednisolone

DR P.J.AUGUST AND DR G.C.WELLS

St Thomas' Hospital, London

F.S., male, aged 52 years.

History. The patient developed pyoderma gangrenosum 6 weeks after prostatectomy in October 1972 for prostatitis which had led to retention of urine. Lesions developed in the suprapubic abdominal wound and numerous ulcers appeared on the legs and trunk with sparing of the arms and face.

On examination. Lesions began as a pustule and enlarged peripherally, becoming a heaped up ulcer discharging copiously from a cribriform base (Fig. 1). There was moderate discomfort. General examination was within normal limits.

Investigations. Skin biopsy from a thigh lesion confirmed the clinical diagnosis. The lesions were bacteriologically sterile. Full investigation failed to show any evidence of ulcerative colitis, Crohn's

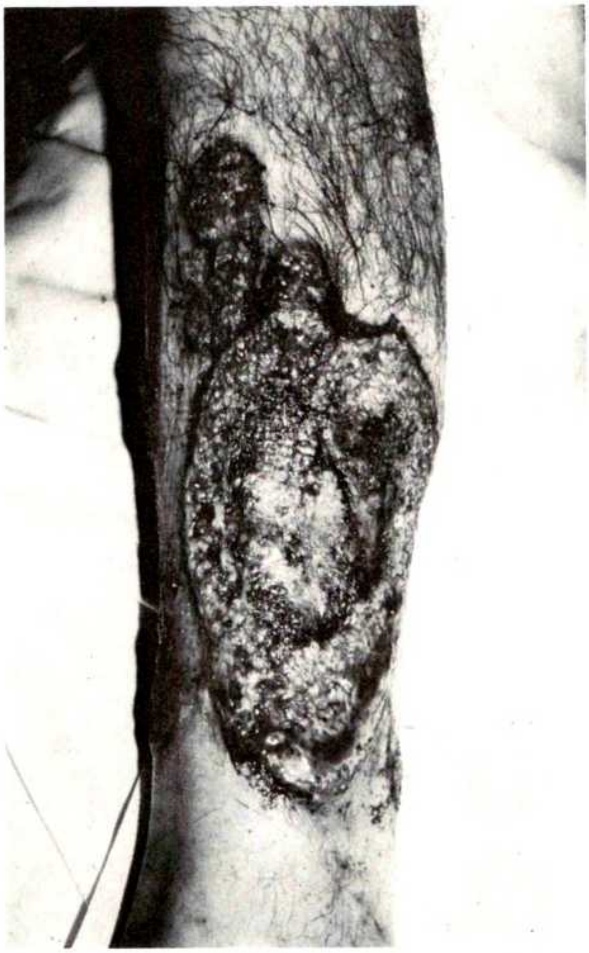

FIGURE 1. Leg lesions at time of presentation in February 1973.

disease, rheumatoid disease or myeloma. Abnormalities were confined to an ESR of 30, a slight rise in plasma IgM levels, which was followed by a fall in IgA levels. In addition, there was evidence of iron deficiency with a low serum iron, a high iron binding capacity and a marrow aspiration which failed to stain positively for iron.

Course and treatment. When he was referred in February 1973 he was already taking prednisolone 30 mg daily, having been treated previously by various antibiotics without improvement. No benefit accrued from raising the prednisolone dose to 80 mg daily and, indeed, he lost 6 kg in weight during the next 3 months. He showed no signs of steroid toxicity although his endogenous production of steroid was suppressed by the prednisolone. Ferrous sulphate given for biochemical iron deficiency was ineffective. Salazopyrine was equally useless, the rationale being that he may have had subclinical ulcerative colitis. A transfusion of two pints of fresh blood did not help (D'Sluis, 1966), nor did intralesional steroid injections (Levene, 1973; Moschella, 1967).

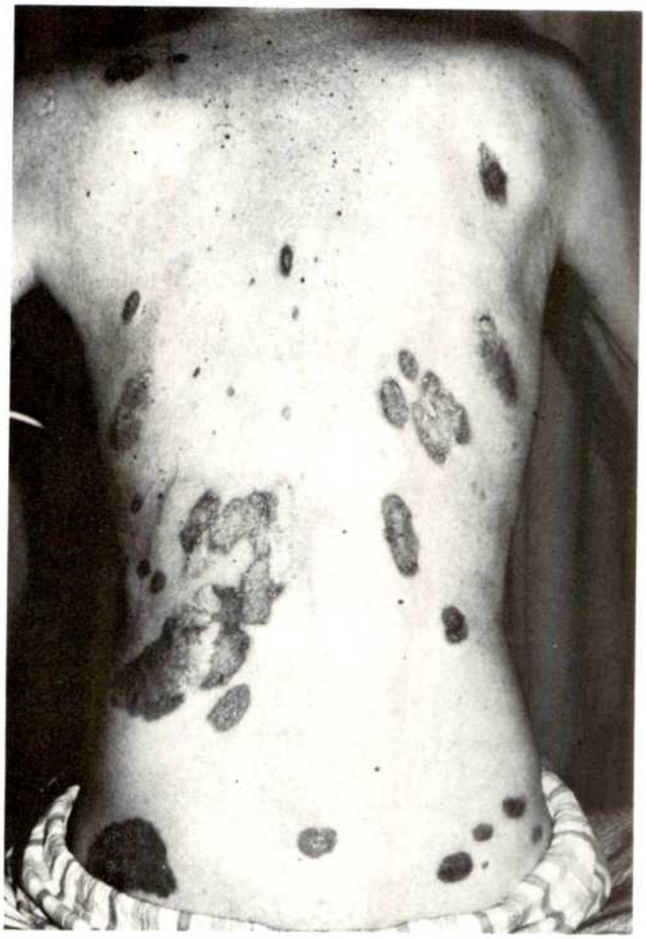

FIGURE 2. Lesions on back after prednisolone and azathioprine, August 1973.

Real improvement occurred in one lesion on the thigh which received 2500 rad of 660 kV X-ray therapy in nine doses over a period of 1 month. Lesser improvement took place after 2000—2600 rad of 70 KV X-ray therapy to other lesions. However, the most impressive improvement began some 3 weeks after starting azathioprine 150 mg daily. He regained 5 kg in weight, the remaining ulcers healed up and no new lesions formed. Side effects consisted of an absolute lymphopenia of 200–400. A short lived attack of epididymo-orchitis cleared up after 1 week's treatment with ampicillin. His prednisolone was reduced from 80 mg to 15 mg daily over a 3 month period. Slight relapse occurred in December 1973 with some new lesions on the buttock. These failed to clear with 70 kV X-ray therapy so his prednisolone was raised to 30 mg a day and then to 40 mg daily with considerable improvement (Fig. 2). In March 1974 a repeat of the plasma protein electrophoresis and urinalysis for Bence-Jones protein were normal, but he continues to be mildly iron deficient.

Discussion. Pyoderma gangrenosum has been reported in association with ulcerative colitis (d'Sluis, 1966), Crohn's disease, rheumatoid disease and myeloma (Rockl, Krede & Schropl, 1964). It is disturbing that one of the myeloma patients reported by Jablonska, Stachon & Dabrowska (1967) had pyoderma which antedated detection of paraproteinaemia by 4 years. It may, therefore, be a difficult matter to be certain that pyoderma gangrenosum is idiopathic.

The use of azathioprine in pyoderma gangrenosum was reported on unfavourably by Corley in 1964 (Corley, Lessner & Larsen, 1966), but Schöpf, Schulz & Schulz (1969) found it to be of value. Mercaptopurine, which is a metabolite of azathioprine, was reported as causing a dramatic remission of pyoderma gangrenosum in a child with acute leukaemia (Maldonedo *et al.*, 1968). Other investigators have used cyclophosphamide (Crawford, Sherman & Favara, 1967) and corticosteroids (Hofstad, 1959; Holt & Waddington, 1973) although they did not help this patient. The use of radiotherapy does not seem to have been reported recently.

In our particular case, a combination of moderately high doses of steroids and azathioprine was essential. High dose steroids, however, were ineffective and once control was acheived a reduction of steroid dosage was followed by relapse.

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